Relationship between intracranial pressure and cervical lymphatic pressure and flow rates in sheep

I. Silver, B. Li, J. Szalai, and M. Johnston. Relationship between intracranial pressure and cervical lymphatic pressure and flow rates in sheep. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1712–R1717, 1999.—Previous reports from our group demonstrated that about one-half of the total volume of cerebrospinal fluid (CSF) removed from the cranial vault in sheep is transported into extracranial lymphatics, especially cervical lymphatic vessels in the neck. In this study, we tested the hypothesis that an elevation of intracranial pressure (ICP) would increase cervical lymphatic pressure and lymph flow rates in anesthetized sheep. Catheters were inserted into both lateral ventricles, the cisterna magna, cervical lymphatics, and the jugular vein. A ventriculo-cisternal perfusion system was employed to regulate ICP. Mean (P = 0.008), peak (P = 0.007), and baseline (P = 0.013) cervical lymphatic pressures increased as ICP was elevated from 10 to 70 cmH2O in 20-cmH2O increments. Similarly, cervical lymph flow rates increased (P < 0.001), with flows at 70 cmH2O ICP observed to be approximately fourfold higher than those at 10 cmH2O ICP. No changes were observed in mesenteric lymph flow rates (vessels not expected to drain CSF). We conclude that cervical lymphatic vessels play an important role in the transport of CSF from the cranial vault when ICP is elevated.

CEREBROSPINAL FLUID (CSF) transports from the cranial vault not only through arachnoid villi into the venous sinuses of the brain but also through the cribiform plate into extracranial lymphatic vessels (reviewed in Ref. 7). Recent studies have demonstrated the quantitative significance of the lymphatic route in resting states. Approximately one-half of the total CSF-to-plasma transport of a protein tracer occurs through extracranial lymphatics in adult sheep (5) and rats (3). Additionally, tracer recovery data in a three-compartment mathematical model have been used to estimate the volumetric CSF clearance into lymphatics. Remarkably, this study demonstrated that about one-half of the total volume of CSF absorbed from the cranial vault was removed by lymphatic vessels (4).

The cervical lymphatic vessels in the neck provide the most important lymphatic pathway for CSF clearance. In this regard, raised intracranial pressure (ICP) elevates the concentrations of CSF protein tracers in cervical lymph nodes (19) and in cervical lymph (2). Indeed, there are reports in the literature suggesting that total cervical lymph flow is associated with elevation of ICP in cats (17), dogs (13), rabbits (20), and sheep (2). However, the relationship between ICP and cervical lymphatic parameters has not been assessed systematically. In addition, the ability of the cervical vessels to transport CSF may be restricted by the nature of the anatomical pathways that deliver CSF to lymph-accessible sites. Bradbury and Westrop (8) have speculated that the greatest resistance to CSF transport to extracranial lymphatics may occur as the CSF moves through the perineural spaces within the rigid bone of the cribiform plate. If this is the case, the ability of cervical vessels to transport CSF in response to raised ICP may be limited.

The purpose of the experiments outlined in this report was to test the hypotheses 1) that cervical lymphatic pressure and flow are related directly to ICP and 2) that the elevated CSF clearance by cervical lymphatics occurs over a wide range of ICPs.

MATERIALS AND METHODS

Randomly bred female sheep weighing 20–40 kg were purchased from LeDo farms (Ontario) for this investigation. They were fed hay, pellets, and water ad libitum but were fasted 24 h before surgery. Experiments were approved by the ethics committee at Sunnybrook Health Science Center and conformed to the guidelines set by the Canadian Council on Animal Care and the Animals for Research Act of Ontario.

Surgical preparation. The sheep were anesthetized initially by intravenous infusion of 5% sodium pentothal solution. After this, the animals were intubated and surgical anesthesia was maintained using halothane administered through a Narkomed 2 respirator. An incision was made in the sheep’s scalp to reveal the junction of the sagittal and lambdoid sutures. Two 1/8-in. burr holes were made bilaterally 1.5 cm anterior and 1.5 cm lateral to the lambda, at an angle of 10° from the sagittal plane. A single catheter guide screw was inserted in each hole. A 16-gauge Novalon intravenous catheter (Becton Dickinson, Sandy, UT) was then attached to a column of filter-sterilized artificial CSF (as described in Ref. 10) and fed through the guide screw. Entry of the catheters into the lateral ventricles was confirmed by a sudden drop in artificial CSF volume in the column. One of the catheters was connected to a raised reservoir filled with artificial CSF, whereas the other was coupled to a pressure transducer (CDXpress, Cole, Lakewood, CO). A laminectomy was performed on C1 to expose the cisterna magna, which was cannulated with a 140-cm-long vinyl catheter filled with artificial CSF (Dural Clear Vinyl Tube; ID 1.00 mm, OD 1.50 mm). The catheter was secured to the dura and exteriorized. ICP was controlled by adjusting the height of the inflow reservoir and the outflow catheter appropriately. An incision was made in the neck and the jugular vein, and cervical lymphatic vessels were exposed. A vinyl catheter (ID 1.0 mm, OD 1.5 mm) was inserted into the jugular vein. The outflow
end of this catheter was connected to a stopcock, one arm of which was in continuity with a pressure transducer (CDX

press, Cobe) for monitoring of central venous pressure (CVP).

In the majority of experiments, ICP and CVP were recorded on two channels of a physiological recorder (Hewlett Packard 7758A recorder). In several experiments, data were recorded on a computer-based data-acquisition system (A-Tech Instruments, Visual Designer Software).

Measurement of lymph flow rates. A cervical lymphatic vessel was cannulated using an 18-gauge Novalon intravene-

nous catheter attached to a three-way stopcock. This, in turn,

was connected to a vinyl catheter (ID 1.0 mm, OD 1.5 mm). In

the event of lymph clotting, the catheter could be flushed with

a heparin saline solution. All side branches, tributaries, and

other cervical lymphatics were tied off. The cervical lymphat-

ics empty into the venous system at the base of the neck and,

therefore, cervical lymph flow is opposed by the CVP. Because

the cervical duct was cannulated and the lymph diverted in

some of the experiments, the normal outflow pressure into

which this vessel transports lymph would be altered. To

maintain the physiological relationships as close as possible
to the natural state, we simulated the outflow pressure

encountered by the cervical lymphatic at its normal lymph-

atic venous anastomosis by adjusting the height of the

lymphatic outflow catheter to create a total outflow resistance
equivalent to the CVP, which was monitored continuously

throughout the experiment (described in Ref. 2). However, in

two of the six animals used for data analysis in this part of the

study, this procedure reduced the cervical lymph flow to zero.

To reestablish flow in these two preparations, the outflow end

of the catheter was lowered 2.5 cm below the measured CVP.

Next, an incision was made in the right abdominal wall, and

the mesentery was exteriorized. The main mesenteric trunk

draining the ileocecal junction was cannulated using a 72-cm

long vinyl catheter (ID 1.0 mm, OD 1.5 mm), and the

mesentery returned to the abdominal cavity. The outflow end

was set at midthoracic height.

The openings of the cervical and mesenteric outflow cath-

eters were placed immediately adjacent to two lever-arm

isometric transducers (Gould Statham model UC-3) that were

connected to one of the channels of a second physiological

recorder (RS11A; Beckman Instruments). The outflow cath-

ders were positioned such that lymph flowed onto the arm of

the transducers. As the drop of lymph formed on the trans-

ducer arm, an increase in tension was recorded. When the

drop fell off the lever, the transducer was automatically reset

(14). The drop counter was calibrated from the cervical and

mesenteric lymph that was collected at 15-min intervals

throughout the experiment.

Measurement of lymphatic pressure. Two vinyl catheters

(15 cm in length, ID 1.5 mm, OD 2.5 mm) were inserted into

the cervical lymphatic, one against the direction of flow and

one downstream in the direction of flow. The outflow end of

the upstream catheter and the inflow end of the downstream

catheter were attached to a plastic t-piece such that cervical

lymph continued to flow in the venous system. A Millar

solid-state pressure transducer catheter (SPR-407, Houston,

TX) was placed through a Cobe sampling plug into the

sidearm of the t-piece. In two of the five experiments designed
to investigate the relationship between ICP and cervical

lymphatic pressure, ICP, CVP, and lymphatic pressures were

recorded on the physiological recorder. In three experiments,

the outputs from the transducers were fed directly to the

computer-based data-acquisition system.

Experimental design. Lymphatic pressures and flows were

assessed at four different ICPs in each animal. The ICP was

set originally at 10 cmH2O (approximate resting ICP in

sheep) and raised incrementally to 30, 50, and 70 cmH2O.

Lymph flow rates and lymphatic and central venous pres-

sures were monitored continuously for 45 min at each pres-

sure.

Data analysis. We assessed the ICP-versus-lymph flow

relationships in eight animals. Mesenteric flow data were

obtained in all sheep, but cervical lymph flow data were

obtained in six animals due to lymph clotting in two sheep.

ICP vs. cervical lymphatic pressure was assessed in six sheep,

but the data from one animal had to be omitted due to the

deposition of fibrin on the Millar transducer tip.

The baseline, peak, and mean lymphatic pressures and

normalized lymph flow rates were plotted over time. Normal-

ization of the lymph flow data was achieved by dividing each

value by the maximum value obtained in that vessel for that

animal. This permitted meaningful comparisons because of

the variability in flow rates measured from vessels of differ-

ent sizes. Mean changes in lymphatic pressure or lymphatic

flow rates as a function of ICP were analyzed through

repeated-measures ANOVA.

RESULTS

Relationship between ICP and cervical lymphatic pressure. In all animals, a rise in ICP was associated with an increase in cervical lymphatic pressure (example illustrated in Fig. 1). In these experiments, the cervical vessels continued to empty into the venous system at the base of the neck. Therefore, a change in

CVP could affect cervical lymphatic pressure by altering

the outflow pressure. However, we did not observe changes in CVP as ICP was elevated in any of the experiments performed (Fig. 1). The mean data from five animals are plotted in Fig. 2. Taking the average of

the last 15 min of each monitoring period, we observed

Fig. 1. Relationship between intracranial pressure (ICP), cervical

lymph pressure, and central venous pressure (CVP) in one animal.
ANOVA revealed significant increases in baseline (P < 0.013), mean (P = 0.008), and peak lymphatic pressures (P = 0.007) as ICP was elevated from 10 to 70 cmH₂O.

As was the case with cervical lymphatic pressure, rates.

and 30 cmH₂O ICP, the change in lymph flow was very

flow rates (example illustrated in Fig. 3). Between 10

a rise in ICP produced an increase in cervical lymphatic

pressure rose only slightly (from 2.58 to 3.60 cmH₂O). However, as ICP was raised from 30 to 50 and 50 to 70 cmH₂O, cervical lymphatic pressure increased to 5.79 and 8.06 cmH₂O, respectively.

Relationship between ICP and cervical lymph flow rates. As was the case with cervical lymphatic pressure, a rise in ICP produced an increase in cervical lymphatic flow rates (example illustrated in Fig. 3). Between 10 and 30 cmH₂O ICP, the change in lymph flow was very small, but as ICP was elevated further, cervical flow rates increased considerably. Similarly, lowering of ICP resulted in a reduction of cervical lymph flow rates (Fig. 4). Mesenteric lymph flow rates monitored in the same animal did not change when ICP was elevated or lowered (Fig. 4 example). Changes in ICP resulted in fairly rapid cervical flow responses. Furthermore, it appeared as though the magnitude of the delay in cervical response became shorter as ICP was elevated. In the example illustrated in Fig. 3, elevation of ICP from 10 to 30 cmH₂O produced little discernable increase in cervical lymph flow rate, but an elevation of ICP to 50 cmH₂O produced a change within ~5 min. The change in ICP from 50 to 70 cmH₂O produced an almost immediate increase in lymph flow rate. In the example illustrated in Fig. 4, ICP was reduced from 70 to 10 and cervical lymph flow decreased within a few minutes. In this same example, raising ICP back to 70 cmH₂O had an almost immediate effect on cervical flows.

Analysis of the mean normalized data (Fig. 5A) demonstrated a significant increase in cervical lymph flow associated with elevation of ICP (P < 0.001). Flow rates increased approximately fourfold as ICP was elevated from 10 to 70 cmH₂O. Absolute flow rates averaged 0.82 ± 0.02, 1.05 ± 0.03, 1.75 ± 0.04, and 3.15 ± 2.32 ml/h at 10, 30, 50, and 70 cmH₂O ICP, respectively. No significant changes in mesenteric lymphatic flow rates measured concurrently were observed (Fig. 5B).

In attempting to determine the rate change of cervical lymph flow rates for a given change in ICP, it appeared as though there were two distinct slopes for the ICP-versus-lymph flow relationship. Between 10 and 30 cmH₂O ICP, the slope was relatively shallow but increased between 30 and 70 cmH₂O. To test the change in the two slopes directly, another ANOVA was performed. The four pressure levels were now defined by two within-subject factors of time (early vs. late) and pressure (low vs. high) within each level of time. As the dependent measure represented a proportion, it was subjected to an arcsine transformation prior to analysis (11). The interaction term (time × pressure), which assesses the difference between the two slopes directly, was statistically significant (P = 0.0246).

DISCUSSION

Relationship between ICP and cervical lymphatic pressure and flow. The data outlined here support the concept of hydraulic coupling between CSF and cervical lymph in the sheep. Incremental changes in ICP were reflected by significant increases in cervical lymphatic pressures and flow rates. In anesthetized cats, cervical lymph flow rates increased as CSF pressures were raised after infusion of artificial CSF into the cisterna magna (17), but the increase was not maintained as the infusion continued. In our studies, cervical flow rates were relatively stable once equilibrium had been reached. This may be due to the better control of ICP afforded by the ventriculocisternal perfusion method employed in our experiments.

With regard to the source of fluid that contributes to the cervical lymph flow response, an increase in ICP could lead to elevation of systemic arterial pressure due to sympathetic discharge (Cushing response). This could augment capillary filtration and increase lymph flow rates globally. In the experiments reported here, we measured cervical and mesenteric lymph flow rates simultaneously under conditions in which ICP was varied from low to high levels. If a systemic effect was present, we would have expected all lymphatic flow
rates to increase, but this was not the case. In Fig. 5, the mesenteric lymph flows at an ICP of 70 cmH\textsubscript{2}O were slightly higher than those at lower ICP levels, but no significant effects of ICP on flow rates were observed in these vessels (Figs. 4 and 5B). Lymphatics draining the intestines are not believed to have an important role in CSF clearance. In contrast, elevations of ICP affected cervical flows markedly. These vessels have been implicated in playing an important role in CSF transport. This suggests that the majority of fluid contributing to the ICP-induced cervical lymph response was CSF derived.

In addition, CVP represents an outflow pressure against which the cervical lymphatics are forced to flow. A change in CVP could affect cervical lymphatic pressure and flow independent of or in conjunction with augmented delivery of CSF to the cervical vessels. Because the cervical vessels were cannulated in the flow experiments and lymph diverted from the animal, any in vivo changes in CVP would not affect lymph flow rates in our study. In the case of the pressure experiments, cervical lymph continued to flow into the venous system, and an increase in CVP could affect lymphatic pressure. However, no changes were observed in CVP over the course of the experiments.

Studies with CSF protein tracers also support the CSF compartment as the source of fluid when cervical lymph flow rates increase after elevation of ICP. McComb et al. (19) raised ICP in rabbits and demonstrated that the recovery of tracer infused into the lateral ventricles was increased in the draining cervical lymph nodes compared with recoveries in control animals. Similarly, in sheep, we observed that the recovery of a CSF protein tracer in cervical lymph increased when ICP was elevated from 10 to 30 cmH\textsubscript{2}O, providing more direct evidence that the augmented portion of cervical lymph transport was CSF derived (2). In this latter study, lymph tracer recovery data in conjunction with mass balance equations based on a three-compartment mathematical model were used to estimate the volumetric transport of CSF into the vessels. These calculations suggested that elevations of ICP resulted in enhanced volumetric transport of CSF into the cervical lymphatic vessels.

On the basis of these observations, we concluded that the increase of cervical lymphatic pressure and lymph flow rates observed when ICP was elevated was due primarily to enhanced CSF transport into the extracranial cervical vessels rather than to ICP-induced systemic perturbations that could affect lymph transport or pressure indirectly.

In our previous study, cervical lymph flow rates averaged 9.1 ml/h in conscious sheep (i.e., CSF-derived
creasing ICP from 70 to 10 cmH₂O caused a cervical response. In the experiment illustrated in Fig. 4, destate flow had been established at high ICP levels, more rapidly. In support of this, once a new steady-

tion of ICP changes in the cervical flow and pressure ment probably accounts for the initial delay in reflec-

tory conduits (15). Alternatively, CSF may exit the perineural space to enter the interstitium of the nasal submucosa. Lymphatic vessels present in this tissue collect and drain away the CSF that has become mixed with nasal interstitial fluid. Clearly, anatomical studies are needed in the sheep before this issue can be resolved satisfactorily. In any case, the time required to saturate the pathways leading directly to the cervical ducts or to the nasal submucosal intermediate compartment probably accounts for the initial delay in reflection of ICP changes in the cervical flow and pressure responses. Once saturated, the response of the cervical vessels to changes in ICP would be expected to occur more rapidly. In support of this, once a new steady-state flow had been established at high ICP levels, lowering ICP resulted in a faster cervical lymph flow response. In the experiment illustrated in Fig. 4, decreasing ICP from 70 to 10 cmH₂O caused a cervical flow change in $-2$-3 min and abruptly increasing ICP back to 70 cmH₂O produced an almost immediate increase in cervical flow.

Cervical lymphatic pressure and flow responses at high levels of ICP. In the rabbit studies of Bradbury and Westrop (8), infusion of artificial CSF at increasing rates into a lateral ventricle reduced the fraction of the CSF protein tracer in cervical lymph. This is in contrast to several other published reports that demonstrated increased transport of a CSF tracer into cervical lymph nodes (19) or cervical lymph (2) when ICP was raised. The highest ICP achieved in the studies of Bradbury and Westrop was 9 mmHg, and it is possible that more CSF tracer would have entered cervical lymph if greater ICPs had been investigated. We could find no evidence for a plateau in the ICP-lymph pressure or flow rate relationships in sheep at least up to an ICP of 70 cmH₂O. From the lowest to the highest ICP tested in this study, cervical lymph flow rates increased on average fourfold. This suggests that the pathways leading to cervical lymphatic vessels in sheep play an important role in the venting of CSF from the cranial vault at high ICP levels. Furthermore, we observed a significant change in the slope of the ICP-versus-cervical lymph flow relationship (Fig. 5A). Between 10 and 30 cmH₂O ICP, lymph flow increased 0.12 ml/h for every 10-cmH₂O increment in ICP. Between 50 and 70 cmH₂O ICP, this increased to 0.70 ml/h per 10-cmH₂O increment in ICP.

There are several mechanisms that could contribute to enhanced CSF transport through cervical lymphatic vessels at high levels of ICP. Bradbury and Westrop (8) speculated that the highest resistance to CSF transport would occur as the CSF passed through the channels of the cribriform plate and that other drainage pathways might be more easily expanded to facilitate CSF clearance when ICPs were elevated. As one possibility, these authors suggested that CSF may be shunted into the subarachnoid space surrounding the spinal cord. In sheep, we identified several nodes in the abdominal cavity and thorax that were positioned along lymphatic routes that drained spinal CSF, with the intercostal and lumbar nodes having the most dominant role (6). After the injection of radioactive protein tracers into lumbar CSF, high concentrations of the tracer were demonstrated in thoracic duct lymph. Nonetheless, even though the bony cribriform plate may have limited capacity to expand, it is possible that the number of open channels through the cribriform plate may increase as ICP is elevated. Not all perineural spaces associated with the olfactory nerves may be open at lower ICPs. The expansion of some of these conduits may require a threshold pressure that is reached only at high ICPs.

Another possibility relates to the contractile properties of the lymphatic vessels. Lymphatics can be modeled as a series of hearts with each pumping unit, or lymphangion, containing an inflow and an outflow valve (1, 16). Lymphangion pressure-volume analysis yields contraction loops similar to those of heart with distinct diastolic and systolic phases. As greater volumes of CSF are delivered to the cervical ducts, the baseline or diastolic lymphatic pressure increases (Fig. 2). An increase in transmural pressure would enhance contractile parameters such as stroke volume as has been demonstrated with in situ lymphatic preparations (16), and the increased contractile performance may facilitate CSF transport.

Perspectives

The ease by which CSF is removed from the cranial compartment (CSF outflow resistance; $R_{out}$) can be calculated using a number of infusion methods...
(reviewed in Ref. 12). In several species including humans, the relationship between ICP and $R_{\text{out}}$ is nonlinear with $R_{\text{out}}$ increasing as ICP is raised until a point is reached at which $R_{\text{out}}$ begins to fall (18). Elevations of ICP could decrease system resistance by expanding CSF pathways within the cranium leading to enhanced CSF transport to absorption sites. In this regard, Butler (9) has suggested that the decline in $R_{\text{out}}$ is due to the formation of increasing numbers of open transendothelial channels through the arachnoid villi. However, this increased CSF delivery would apply not only to arachnoid villi but also to sites accessible to the cervical lymphatic vessels. It is of interest to note that in the studies of Mann et al. (18), the $R_{\text{out}}$ declined in humans, dogs, cats, rabbits, and rats as ICPs were elevated beyond 30 cmH$_2$O. In our study, the change in the slope of the ICP-versus-cervical lymph flow relationship appeared to occur somewhere between 30 and 50 cmH$_2$O. At this point, the ability of cervical lymphatics to transport CSF appeared to increase. Therefore, it is possible that enhanced lymphatic transport of CSF could contribute to the decline in $R_{\text{out}}$.

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