Reflex Modulation of Lymphatic Pumping in Sheep

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Lymphatic pumping activity was examined in halothane-anesthetized sheep. A doubly cannulated preparation of the mesenteric lymph duct was “isolated” from lymph input, other than that from a constant pressure reservoir of artificial lymph attached to its inflow cannula, but had its blood supply and innervation intact. A cerebral ischemic response, evoked by injection of 2 ml air into the common carotid artery, increased both mean arterial pressure and fluid propulsion by the lymphatic. The latter rose from a control value of 45 μl/min to a peak of 74 μl/min. When 10^-4 M phentolamine was introduced into the lymphatic lumen, there was a transient increase followed by a sustained fall in lymph pumping. Repetition of the air injection while phentolamine was present in the duct lumen produced no increase in lymph pumping. In adrenalectomized animals, resting lymph propulsion by the mesenteric duct was depressed, and the response to air injection was attenuated but remained significantly greater than control. These results suggest that reflex activation of the sympathetic nervous system can increase lymph propulsion and that this may be augmented by the release of circulating catecholamines. (Circulation Research 1989;64:1165-1171)

Intrinsic contractions of the lymph vessels of many mammals, including humans, contribute much of the energy for lymph propulsion, yet little is known about the physiological control mechanism that might regulate their pumping action. It is known that lymph vessels have a noradrenergic innervation1 and that stimulation of the intramural nerves in isolated segments of bovine mesenteric lymphatics increases the frequency of their spontaneous contractions2 and increases fluid propulsion by cannulated segments. However, it is not yet clear what role, if any, these nerves might play in the control of lymph flow in a living animal. Recently, McGeown et al3 have shown that stimulation of the lumbar sympathetic chain increases hind limb lymph flow in the sheep. While this study demonstrates the existence of a functional innervation in the living animal, it does not distinguish between effects of sympathetic stimulation on lymph formation from those on lymphatic contractility nor does it demonstrate that activation of the animal’s own autonomic nervous system modifies lymph flow. Power and Brace4 have suggested that the autonomic nervous system has no role in modification of lymphatic contractility since the autonomic ganglion blocker hexamethonium did not modify lymph flow under a variety of conditions. In the present study, we have examined the possibility that intense reflex activation of the sympathetic nervous system might modify the activity of the intrinsic lymph pump in the anesthetized sheep.

Arterial air embolism is known to produce large transient increases in arterial pressure in humans,5,6 and it seemed that this might be a convenient way of evoking a strong sympathetic response in the sheep. When it was established that the injection of 2 ml air into the carotid artery could repeatedly elicit such a response, the effect of this procedure on pumping activity of the doubly cannulated sheep mesenteric lymphatic preparation developed by McHale and Thornbury7 was examined. The results suggest that reflex activation of the sympathetic nervous system may indeed play a physiological role in the control of lymph flow.

Materials and Methods

Ewes (30–45 kg) that had been fasted for 48 hours were anesthetized with pentobarbital (20–30 mg/kg i.v.) and maintained with 2–3% halothane in O₂. The mesentery was approached through a paramedian incision, and enough of the small intestine was exposed to allow location of the main mesenteric lymph duct. This was cannulated at both ends with silicone rubber tubing (0.047 in. o.d., 0.025 in. i.d.). Evans blue dye was then injected into the lymph...
nodes to locate branches joining the cannulated segment so that these could be tied off. The inflow cannula was connected via a four-way junction to a reservoir of “artificial lymph” that had previously been prepared by diluting the animal’s own plasma with an equal volume of isotonic heparinized saline. The other arms of the junction were connected to a reservoir containing phenolamine dissolved in “artificial lymph” and to a drain tap. The junction was placed close to the lymphatic so that artificial lymph flowing through the vessel could be changed quickly to that containing the α-adrenergic blocker phenolamine. The outflow cannula was connected via a Cobe CDX3 disposable pressure transducer to a flow measuring device similar to that described by McHale and Roddie. This consisted of a force displacement transducer (model FT03, Grass Instrument Co, Quincy, Massachusetts) with a small piece of filter paper attached to its lever. The outflow tube was positioned by means of a micromanipulator so that a fluid bridge formed between its tip and the filter paper. Fluid could thus accumulate on the transducer arm with a minimum of surface tension artifact so that the tension measured was an accurate reflection of the volume of fluid leaving the cannula. When enough fluid had accumulated to form a drop, the drop fell off and the voltage output was reset to a new level. Via a cannula in the right femoral artery, blood pressure was measured with a second Cobe disposable pressure transducer. The output from both pressure transducers and from the force displacement transducer was recorded on a polygraph (model 7D, Grass). In adrenalectomized animals, this was achieved in a two-stage operation in which each adrenal was approached in turn via a lateral abdominal incision. The adrenal was located in the retroperitoneal space superior to the kidney and was

gradually mobilized by blunt dissection, its vascular and nerve supply was tied off or cauterized, and the entire gland was removed.

The experimental protocol consisted of an equilibration period during which the lymphatic began to pump artificial lymph. When pumping became regular, 2 ml air was injected into the common carotid artery. In experiments in which blocker was used, artificial lymph containing phenolamine was substituted for the control perfusate 30 minutes after the air injection. A further 30 minutes was then allowed for flow to stabilize, and the air injection was repeated. In some experiments, 0.2 ml norepinephrine (1 mg/ml) was injected into the femoral vein to confirm that intraluminal blocker was indeed effective or to assess the lymphatic responsiveness to norepinephrine after adrenalectomy. The statistical significance of any changes noted was assessed by comparing the raw data using Student’s paired t test or repeated measures analysis of variance in the case of the mean frequency and flow before and after air injection in the absence or presence of phenolamine. Drugs used were L-norepinephrine bitartrate (Levophed, Winthrop-Breon Laboratories, Division of Sterling-Winthrop, New York, New York) and phenolamine mesylate (Regitine, CIBA Pharmaceuticals, Division of CIBA-GEIGY Corporation, Summit, New Jersey).

Results

Thirty sheep were used in this study. Of these, 16 were judged satisfactory. The others were rejected because the side branches to the cannulated lymphatic could not be tied off completely (this was checked by clamping off the inflow tube whereupon, in successful preparations, flow ceased within 10–15 minutes?) or because, for a variety of reasons, the lymphatic was not pumping fluid regul-
larly. In experiments that were considered satisfactory according to the above criteria, a record such as that shown in Figure 1 was obtained. Each time the lymphatic contracted, there was a phasic increase in outflow pressure, and this resulted in the expulsion of fluid onto the transducer arm (seen as a small step in the ramp in the middle trace). When enough fluid had accumulated to form a drop, this fell off, and the process was repeated forming a series of ramps the slopes of which were an index of flow. Since flow in this preparation is known to be very sensitive to distending pressure, this was standardized at 5 cm H2O. Under these conditions, flow was reasonably constant for any one preparation, but there was considerable variation from animal to animal with control flows ranging from 22 to 67 μl/min and averaging 44±13 (SD) μl/min (n=8).

**Effect of Air Injection**

When 2 ml air was injected into a common carotid artery, heart rate and respiratory rate tended to fall, and there was sometimes a transient fall in systemic arterial pressure (an example of this transient fall can be seen in Figure 4). This was followed within 1 minute by a large rise in arterial pressure. In the experiment shown in Figure 1, mean arterial pressure rose from 89 mm Hg before air injection to a peak of 133 mm Hg within 3 minutes of the injection and then gradually declined to control level over the next 10 minutes. Control lymph flow averaged 37 μl/min. This increased to a peak of 47 μl/min 2 minutes after air injection and then gradually declined to control level within 15 minutes. In eight experiments, the peak response varied from a 23% to a 90% (mean 55%) increase over control.

A summary of the eight experiments is shown in Figure 2. Each point in the upper panel is the average of mean arterial pressure at the end of fifteen 1-minute periods. Each point in the middle and lower panels is the mean of lymphatic contraction frequency and lymph flow (respectively) averaged over the preceding minute. After air injection, average mean arterial pressure increased from a control value of 90 mm Hg to a peak of 124 mm Hg within 3 minutes and then gradually decreased towards control. Average frequency increased from five contractions per minute to a peak of 6.5 per minute while lymph flow increased over the first 4 minutes after air injection from a control value of 45 μl/min to a peak of 74 μl/min and declined, rapidly at first, then more slowly over the next 6 minutes.

**Effect of Phentolamine**

Figure 3 shows the effect of changing the fluid being pumped through the lymphatic from artificial lymph to a similar fluid containing 10^-4 M phentolamine. Before changing the perfusion fluid the lymphatic was contracting at a frequency of three per minute and pumping fluid at a flow rate of 39 μl/min. Shortly after the introduction of phentolamine, lymphatic frequency increased to 8.9 contractions per minute and flow to 48 μl/min. This excitatory effect is typical of phentolamine (due to its action as a partial agonist) on isolated bovine mesenteric lymphatics (McHale and Thornbury, unpublished observation) and usually lasts for 10–20 minutes in the isolated vessels, and then activity returns to control levels. In the present preparation, however, when the direct transient excitation wore off, flow fell to levels lower than control and frequency rose to a higher level. In seven experiments, the mean flow before phentolamine was 42±4.8 μl/min. When flow stabilized after the transient excitation due to phentolamine, it had fallen to 27±6.5 μl/min. This was significantly different when compared by repeated measures analysis of variance (ANOVA) (Fisher’s least significant difference test) from any of the five control points.
was added to the artificial lymph. The doubling in lymph flow in response to norepinephrine was abolished by intraluminal blocker.

Figure 4 shows an experiment in which the effect of air injection was examined before and after the addition of $10^{-4}$ M phentolamine to the artificial lymph being pumped through the lymphatic lumen. In the lefthand panel, the duct was pumping fluid at a flow rate of 51 $\mu l$/min. Within 2 minutes of air injection, this had risen to a peak of 136 $\mu l$/min, after which it declined to control. When the air injection was repeated in the presence of phentolamine, there was little or no increase in flow. Mean arterial pressure, on the other hand, increased from 89 mm Hg to a peak of 130 mm Hg before phentolamine and from 78 mm Hg to a peak of 115 mm Hg after repetition of the air injection. Figure 5 shows a summary of seven experiments in which the effect of air injection was examined in the presence of $10^{-4}$ M phentolamine. Before air injection, lymph flow averaged 28 $\mu l$/min. There was a slight decline in flow during the first 4 minutes after the injection, but when average flow before and after injection were compared by ANOVA, the decrease was not found to be significant. Although there was not clear evidence of the reversal of an $\alpha$-excitatory effect to a $\beta$-inhibitory one such as that found with isolated bovine vessels,2 the flow increase was clearly blocked.

It could be argued that the lymph flow response to cerebral ischemia disappeared when the injection was repeated. To exclude this possibility, four experiments were performed in which air injection was repeated in the absence of phentolamine. The mean lymph flow was 24±5.5 $\mu l$/min before the first air injection increased it to a peak of 39±11.6 $\mu l$/min. When the air injection was repeated, lymph...
Mean Arterial
Pressure
mm Hg

Frequency
beats/min

Lymph Flow
µl / min

FIGURE 5. A summary of seven experiments such as that shown in Figure 4. The method for calculating each point was as in Figure 2. Air injection still provoked a rise in mean arterial pressure but lymph propulsion was unaffected or slightly depressed.

Effect of Adrenalectomy

In adrenalectomized animals lymph flow was lower and more variable than in animals with intact adrenals. Control flow ranged from 5 to 44 µl/min averaging 26±17 µl/min (n=5), and the effects of air injection on blood pressure and lymph flow were attenuated but still evident. Figure 6 shows one such experiment. Before air injection, lymph flow was 37 µl/min, whereas after the injection, this increased to a peak of 50 µl/min. Over the five animals studied, peak flow increases ranged from 18% to 85% of control with a mean of 38%. When average flow for the 5-minute period before air injection was compared by a paired t test with that of the 5-minute period after injection, the difference was significant (p<0.01). The lymphatic response to intravenous norepinephrine was greatly attenuated in the adrenalectomized animals. A 0.2-ml injection of norepinephrine (1 mg/ml) caused a mean flow increase from 23±14 to 47±29 µl/min (p<0.01, n=6, paired t test) in animals with intact adrenals, while the same dose caused an increase from 29±12 to 36±17 µl/min (NS, paired t test, n=4) in the adrenalectomized animals.

Discussion

This study establishes for the first time that the autonomic nervous system can exert a direct influence on the pumping activity of lymphatic vessels both tonically and as a reflex response to cerebral ischemia. Much previous evidence suggested that this might be so. Lymphatic vessels respond to catecholamines by increasing their pumping activity both in vitro9,10 and in vivo.8,11-13 They have a noradrenergic innervation,1 stimulation of which increases frequency of spontaneous contractions of isolated strips2 and of lymphatics in living animals,14-16 and lymph flow in conscious animals increases during emotional stress.7,8,17 However, persuasive this circumstantial evidence might appear, the results of a study by Power and Brace4 suggested that the autonomic nervous system makes no contribution to lymph flow regulation. These
authors found no significant differences in basal lymph flow or the increased lymph flow following the administration of 15 mg/kg i.v. hexamethonium. This observation, however, contradicts an earlier study by Shim et al.\(^\text{18}\) in which they observed that a dose of 2.5 mg/kg i.v. significantly depressed (50%) canine thoracic duct lymph flow. The main difference between the two studies was that the latter authors used unanesthetized animals. There are many instances in the literature where responses to pressor stimuli have been reduced by general anesthetics. For example, in cats halothane almost totally abolished the pressor response to carotid occlusion,\(^\text{19}\) while in dogs there was a greater than 50% reduction in the response to carotid sinus hypotension.\(^\text{20}\) Barbiturate anesthesia caused a 60% depression of the response to carotid occlusion in dogs\(^\text{20}\) and in man abolished the vasomotor response to hypotension during lung inflation.\(^\text{21}\)

The apparent contradiction between the results of Power and Brace\(^\text{4}\) and those of the present study may be accounted for by several factors. The lymphatic system of ruminants, of guinea pigs, and of humans shows clear cut rhythmic contractility, whereas this has not been convincingly demonstrated in the dog. One might expect a difference, therefore, in the responses of canine lymphatics to sympathetic stimulation. This would not mean, however, that there would be no response to sympathetic stimulation (see Shim et al.\(^\text{18}\)) since dog lymphatics are certainly contractile. Another possible reason for the differences between the present study and that of Power and Brace\(^\text{4}\) is that the anesthetic used was pentobarbital, which is a more potent inhibitor of lymphatic contractility than halothane,\(^\text{22}\) so, quite apart from any depressant effects that the anesthetic might have had on reflex responses, lymphatic contractility may have been depressed by their use of this anesthetic throughout their experiments. It may also be that, even if lymphomotor reflexes in response to less severe stimulation of baroreceptors and volume receptors do exist, it is not possible to demonstrate them in anesthetized animals. For example, Thornbury\(^\text{23}\) found that neither bilateral carotid occlusion nor increases in intrathoracic pressure had any significant effect on hind-limb lymph flow in anesthetized sheep, whereas there have been several demonstrations of increases in lymph flow in response to stress in conscious animals.\(^\text{7,8,17}\) More extreme stimuli such as that reported in the present study or hemorrhage\(^\text{24}\) (without volume preloading) may be necessary to demonstrate lymphomotor responses in anesthetized animals.

The use of arterial air embolism to evoke a sympathetic response could be regarded as a crude approach since there was no way of predicting exactly where the air would go and which areas of the brain would be rendered ischemic. We feel, nevertheless, that its use was justified in that it consistently produced a large increase in arterial pressure after many repetitions. On one occasion, it was repeated seven times at 30-minute intervals. The first air injection caused mean arterial pressure to rise from 97 to a peak of 138 mm Hg, a rise of 42%. After six air injections, the resting mean arterial pressure was 73 mm Hg and the seventh air injection caused this to increase to a peak of 96 mm Hg, a rise of 31%. In fourteen experiments the effect of a second air injection on peak mean arterial pressure was compared with that of the first. The average mean arterial pressure before the first injection was 88.2±2.4 mm Hg. This rose to a peak of 131±6.8 mm Hg after the first injection. Thirty minutes later, after the second air injection, the pressure rose from 83±3.4 to 126±4.8 mm Hg. Both of these rises were significant (ANOVA repeated measures, Fisher’s least significant difference test, 95% level) while the difference between the peak mean arterial pressure response evoked by the first and second injections was not significant.

The results in the adrenalectomized animals would suggest that circulating catecholamines play a major role in the enhanced pumping response after air injection. This may be a superficial interpretation, however, since adrenalectomy is known to decrease responsiveness of the entire cardiovascular system to adrenergic stimulation.\(^\text{25}\) Thus, hypotension following adrenalectomy is a function not only of the decreased catecholamine output but also of the absence of the “permissive effect” of the adrenal cortical hormones,\(^\text{26}\) and corticosteroids are known to potentiate the action of norepinephrine and epinephrine on vascular smooth muscle.\(^\text{27-29}\) These observations are relevant to the present study because the increase in lymph propulsion caused by intravenous injection of norepinephrine in the adrenalectomized animals was only 25% of that observed in the intact animals.

Why there should be a reflex lymphatic response such as that described here is not entirely clear, but one possible role for such a mechanism might be to facilitate the return of protein and fluid to the blood vascular system in circulatory emergencies such as hemorrhagic shock. The pattern of lymphatic response to arterial air embolism reported here is, in fact, very similar to the initial increase in hind-limb lymph flow following hemorrhage in conscious sheep described by McHale and Thornbury\(^\text{30}\) except that there was a somewhat greater response in that study. Those results were difficult to interpret, however, since the conscious animals tended to move more after blood withdrawal, and movement is known to increase hind-limb lymph flow. Hayashi et al.\(^\text{34}\) used the same doubly cannulated mesenteric duct preparation that was used in the present study and reported a twofold increase in fluid pumping within 15 minutes of the beginning of blood withdrawal. This response was again considerably greater than that reported in the present study, but this is not surprising when one considers how complex a stimulus hemorrhage is.
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References

KEY WORDS • lymph flow • sympathetic nervous system • adrenalectomy
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