PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 805-807 (1975)

Effects of Histamine and Acetylcholine on Equine Digital Lymph Flow and Composition[1] (38903)

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Laminitis is a common clinical condition affecting the feet of horses and is characterized by heat in the digit and frequently by edema at the coronary band (the junction of the hoof with the skin). It is well known that local administration of histamine causes increased blood flow and edema in many species and histamine has been suggested as a factor in the etiology of laminitis (1). We have recently shown that local administration of histamine increases equine digital blood flow (ms. submitted for publication), and in this paper we examine its effect on lymph production and protein concentration.

Methods. Eight ponies (107–161 kg) were anesthetized with thiamylal sodium (Surital, Parke-Davis, 10 mg/kg) given intravenously, and anesthesia was maintained with sodium pentobarbital. Using a pressure-cycled ventilator (Bird Mark 9), ponies were ventilated with 40%–60% oxygen in air via an endotracheal tube in order to avoid the hypoxemia which may develop in anesthetized horses breathing air (2). Peak inflation pressure was 25 cm H_2O and respiratory rate was adjusted to maintain the end-tidal fraction of carbon dioxide at 5%–6% as recorded with an infrared analyzer (Beckman LB-2). Ponies were anticoagulated with heparin (500 units/kg).

With the horse in lateral recumbency, a side branch of the median artery of the down forelimb was cannulated just proximal to the carpus for the purpose of drug infusion. A 10-cm incision was made on the medial side of the mid-metacarpal region, and a lymphatic was isolated and cannulated with polyethylene tubing (PE 10) proximal to the first lymph node. Usually, we cannulated a large lymphatic immediately overlying the medial palmar vein. Lymph was collected for 10-min periods in graduated pipets sealed at the lower end. After collection, tubes were sealed and stored in a freezer until analyzed.

Systemic arterial pressure (Part) was recorded from a cannula in the facial artery. Small-vein pressure (Psv) was recorded from a vessel in the venous plexus at the junction of the hoof and the skin. The small-vein cannula was directed upstream and we assumed it recorded a lateral pressure because blood could be withdrawn freely, and pressure decayed rapidly after a bolus injection of saline into the catheter. All pressures were recorded continuously with transducers calibrated daily against a mercury manometer. Thus, the limb was left undisturbed except for three skin incisions to allow cannulation of an artery, a vein, and a lymphatic.

Initially, two 10-min lymph collections were made and the second sample was used as a control. We infused acetylcholine chloride (10.3 μg/min) into the median artery using a Harvard infusion pump. Once Psv reached steady state, we collected two 10-min samples of lymph. This was followed by a further 10-min control sample. Histamine diphosphate was then infused at 10.3 μg/min and when Psv became stable, we collected six 10-min lymph samples during the infusion. The dose of histamine was chosen because in a previous study (ms. submitted for publication) we found that this produced the maximum vascular effect. Both acetylcholine chloride and histamine diphosphate were diluted to 10 μg/ml in isotonic saline solution and infused at 1.03 ml/min. Acetylcholine chloride contained 80% acetylcholine base and histamine diphosphate contained 37% histamine base.

Total protein concentration was measured by a photometric method (3). Albumin and globulin fractions were determined by electrophoresis.

[1] This study was sponsored in part by a grant from the Michigan Horse Show Association and the Morris Animal Foundation.

All data was analyzed by the Student t test modified for paired replicates. $P < 0.05$ was taken as the limit of statistical significance.

Results. Mean vascular pressures, lymph flow, and lymph protein concentration are shown in Fig. 1.

Vascular pressures. Systemic arterial pressure which initially averaged 122 mmHg (range 80–152 mmHg) was unaffected by the local infusion of either acetylcholine or histamine. Small-vein pressure, which initially averaged 29 mmHg (range 18–42 mmHg), increased significantly during acetylcholine infusion, decreased during the second control period, and increased again and remained elevated during histamine infusion. The small-vein pressure became more elevated after 40 min of histamine infusion, at which time the experimental limbs were grossly edematous distal to the point of drug infusion.

Lymph flow. Control lymph flow averaged 0.068 ml/10 min (range 0.005–0.25 ml/10 min). Acetylcholine did not significantly increase lymph flow but histamine caused an immediate increase in flow to an average of 0.47 ml/10 min. Similar high flows were sustained for the duration of the histamine infusion.

Lymph protein concentration. Lymph protein concentration averaged 3.11 g/100 ml (range 1.89–4.63 g/100 ml) during the control period and was unaffected by acetylcholine infusion. Histamine produced a significant increase in lymph protein concentration to an average of 4.67 g/100 ml during the second 10-min collection period. This rise was sustained until the final period when average protein concentration decreased. Although lymph protein concentration decreased between the penultimate and final sample in all except one horse, it remained elevated above control in six of seven animals. The rise in lymph protein was shared between the albumin and globulin fractions and there was no significant change in the albumin/globulin ratio which averaged 0.75 during the control period.

Discussion. These studies show that local administration of histamine to the equine digit increases lymph flow and protein concentration, and produces edema. On the other hand, acetylcholine, also a vasodilator, has no effect on either lymph flow or protein concentration. This is similar to the effect of these two agents in the canine forelimb (4, 5) except that in the dog, acetylcholine slightly but significantly increases lymph protein concentration (4).

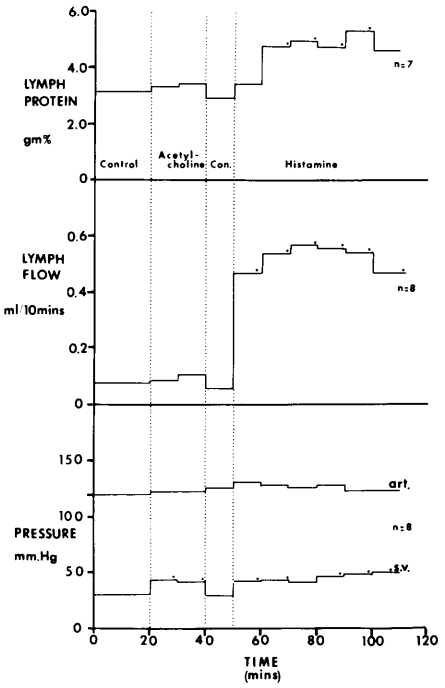

FIG. 1. Systemic arterial pressure (Part), small vein pressure (Psv), lymph flow, and lymph protein concentration during local intraarterial acetylcholine (10 μg/min) and histamine (10 μg/min) infusion. * Indicates a significant difference from the control values recorded during the second 10 min period.

It has been suggested that the mechanism of edema formation in the dog forelimb after histamine infusion is due primarily to a decrease in the transcapillary colloid osmotic pressure gradient and to a lesser degree to an increased transcapillary hydrostatic pressure gradient. The decreased colloid osmotic pressure gradient results from an increase in microvascular permeability to protein generated via both pressure-dependent and pressure-independent mechanisms (4). In the present study, acetylcholine raised small-vein pressure to the same degree as did histamine and in an earlier study (ms. submitted for publication), we showed that both drugs

produced similar increases in blood flow to the equine digit. Since capillary hydrostatic pressure cannot be less than small vein pressure, it is probable that both agents elevated capillary hydrostatic pressure. However, since neither lymph flow nor protein concentration were affected by acetylcholine, it seems unlikely that this rise in capillary pressure was sufficient to greatly alter transcapillary fluid filtration. Infusion of histamine produced large increases in both lymph flow and protein concentration; therefore, our data are consistent with the concept that histamine edema results primarily from a decreased colloid osmotic pressure gradient rather than an increased hydrostatic pressure gradient. The decreased colloid osmotic gradient may result from either increased protein leakage through enlargement of existing pores (6, 7) or increased microvascular protein transport (8). Moreover, our data show that the effect of histamine on lymph flow is immediate and sustained for at least 1 hr. It is also probable that histamine had an immediate effect on protein movement across the capillary. However, this failed to be reflected in a change in protein concentration probably because resident interstitial fluid and lymph had to be expressed from the interstitial tissue, lymphatics, and the cannula.

Another interesting finding in this study is the relatively high protein concentration in the control lymph sample (3.11 g/100 ml) with an albumin/globulin ratio of 0.75. By contrast, lymph protein concentrations in the dog forelimb have been reported to average 2.06 g/100 ml with 1.45 g/100 ml albumin and 0.61 g/100 ml globulin (4). This suggests that equine digital capillaries are more permeable to protein, especially globulin, than are dog forelimb capillaries. However, this difference might be related to the fact that the lymph from the dog forelimb was from both skin and muscle lymphatics whereas in the present study, lymph came from a muscle-free organ whose soft tissue seems to be primarily skin and subdermal tissue.

Thus, local administration of histamine can cause increased blood flow, increased lymph flow and protein concentration, and on prolonged administration, edema. As edema and heat are cardinal signs of laminitis, and as it is thought that the heat is due to increased blood flow, our study is compatible with the suggestion that histamine is a factor in the etiology of laminitis.

Summary. We measured the flow rate and protein concentration of lymph collected from a digital lymphatic in eight anesthetized ponies. Additionally, we recorded systemic arterial pressure (Part), and small vein pressure (Psv). Control lymph flow averaged 0.068 ml/min, and contained 3.11 g/100 ml of protein with albumin/globulin ratio of 0.75. Twenty-minute local intra-arterial infusion of acetylcholine (10 μg/min.) elevated Psv but did not increase lymph flow rate or protein concentration. A 60-min local intra-arterial infusion of histamine (10 μg/min) produced a marked sustained increase in Psv and both lymph flow and protein concentration. Edema developed in the digit receiving histamine. These data support the conclusion that in the horse, as in other species, histamine edema is due primarily to a decreased transcapillary colloid osmotic pressure gradient rather than an increased transcapillary hydrostatic pressure gradient.

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Received Feb. 13, 1975. P.S.E.B.M., 1975, Vol. 149.