Lymphology 23 (1990) 209-214

MESENTERIC LYMPHATIC VASOMOTION FOLLOWING HEMORRHAGE AND RETRANSFUSION IN THE RAT

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ABSTRACT

The effect of hemorrhage and retransfusion on the rhythmic contraction of mesenteric lymphatic vessels was studied in 24 rats, anesthetized with pentobarbital. The rats were divided into four groups according to the amount of blood withdrawn: 0.5ml/100g body weight (BW), 1ml/100g, 2ml/100g, and 2.5ml/100g. Immediately following hemorrhage at the rate of 0.5ml/100g/min, lymphatic contraction frequencies were decreased to 67±12.5, 45±24.7, 43±33.1, and 31±17.8% of the prehemorrhage values in each of the above four groups, respectively ($p<0.01$). Twenty minutes after hemorrhage, lymphatic contraction frequencies were decreased to 70±17.2, 46±36.8, and 34±41.3% in the 0.5ml/100g, 2ml/100g, and 2.5ml/100g, respectively ($p<0.05$). Immediately following hemorrhage, the lymphatic contracted diameters were also reduced to 77±9.7 and 61±9.0% of the prehemorrhage values in the 1ml/100g and 2.5ml/100g groups, respectively ($p<0.01$). Twenty minutes after hemorrhage, all withdrawn blood was reinfused. Lymphatic contraction frequency and contracted diameter recovered after retransfusion in each group but 20 minutes after retransfusion, the lymphatic contraction frequency in the 2ml/100g group was still decreased to 42±30.3% ($p<0.01$). Lymphatic contraction frequency not only decreased proportionately with hypotension during hemorrhage but after retransfusion contraction frequency correlated directly with the mean arterial pressure 20 min after hemorrhage. These data suggest that mean arterial pressure and by inference capillary blood flow and tissue oxygenation are major factors regulating lymphatic vasomotion.

Baez observed (1) that lymphatic contractions in the rat mesentery were more frequent and intense after hemorrhage. No detailed study, however, has been done on the relationship between blood loss and arterial pressure on mesenteric lymphatic contractility and accordingly these interactions have been examined in this report.

MATERIALS AND METHODS

Experimental preparation

Male Wistar rats, weighing 108±20g (mean±SD) were anesthetized by intramuscular injection of sodium pentobarbital (NEMBUTAL®) at a dose of 3.5mg per 100g of body weight (BW). The femoral artery was cannulated to allow withdrawal and subsequent reinfusion of blood, and to monitor systemic blood pressure. This cannula was filled with a heparinized Ringer's solution and connected to a pressure transducer. Observation of mesenteric lymphatic vessels was performed as described previously (2,3). In brief, after 15-20 min, an incision of

about 2cm was made in the lower abdomen. A loop of jejunum of 7 to 10cm in length was gently exteriorized and covered with moist cotton. Thereafter, the rat was placed on a microscope stage in the lateral position and the mesentery was spread on a specially designed plastic block. The mesentery was perfused with mammalian Ringer's solution at 37°C with a pH of 7.4 (4,5). Using a TV camera attached to the microscope, mesenteric lymphatic contractions were observed and recorded on videotape with time markers for later analysis (2-6). Lymphatic contractions were measured usually at the site between the second and third valve. The contraction frequency and the relaxed and contracted diameters were calculated from video reproductions.

In eight rats, 40 min after induction of anesthesia and 20 min after isolation of jejunal loop, the mesenteric lymphatic contraction frequency and relaxed and contracted lymphatic vessel diameter were measured each minute for 60 min.

Hemorrhage

After experimental preparation described above, 24 rats were divided into four groups and blood volume withdrawn to equal 0.5ml/100g, 1ml/100g, 2ml/100g, and 2.5ml/100g BW at the rapid rate of 0.5ml/min by blood withdrawal into a 1ml syringe over a 1 min, 2 min, 4 min, and 5 min interval in the four groups, respectively. The rats were then observed for an additional 20 min period following hemorrhage. The lymphatic contraction frequency and diameters of the mesenteric lymphatics and arterial pressure were recorded at the following time periods: (a) pre-hemorrhage—a 3 min interval before blood loss; (b) during hemorrhage; (c) post-hemorrhage (H-1), a 3 min interval promptly after blood loss; (d) post-hemorrhage (H-2), a 3 min interval 18-20 min after blood loss.

Data were calculated as the mean value per minute in each period and expressed as a respective percentage of the corresponding pre-hemorrhagic value.

Blood reinfusion

In each group of rats 20 min after hemorrhage all withdrawn blood was reinfused, at a uniform rate of 0.5ml/100g BW per min, and observations continued for another 20 min. Data were compiled for 3 min promptly after retransfusion (R-1) and 18-20 min after retransfusion (R-2).

Statistics

The changes during hemorrhage, post-hemorrhage, and post-retransfusion were compared to the pre-hemorrhagic values using student's paired t test. The difference among the means of each group was evaluated using analysis of variance (ANOVA). Differences based on p values of 0.05 or less were considered significant. Data are shown as the mean value and standard deviation (SD).

RESULTS

After anesthesia and dissection, mesenteric lymphatic contraction frequencies were unstable during the initial 17 min; however, they stabilized after 18 min (i.e., 58 min after anesthetic injection), and remained stable for a subsequent 43 min *(Fig. 1)*. The relaxed and contracted lymphatic diameters were largely unchanged throughout. The mean arterial pressure was 70 to 100mmHg.

Hemorrhage

During the period of hemorrhage and fall in blood pressure, the lymphatic contraction frequency, and contracted and relaxed diameter in each group did not change significantly from the pre-hemorrhage value. The absolute value of lymphatic contraction frequencies in the period before hemorrhage for the four groups was 9.4±4.19/min. Promptly after hemorrhage (H-1), lymphatic contraction frequencies decreased to 67±12.5, 45±24.7, 43±33.1, and 31±17.8% in the 0.5ml/100g (n=6), 1ml/100g (n=6), 2ml/100g (n=6), and 2.5ml/100g (n=6)

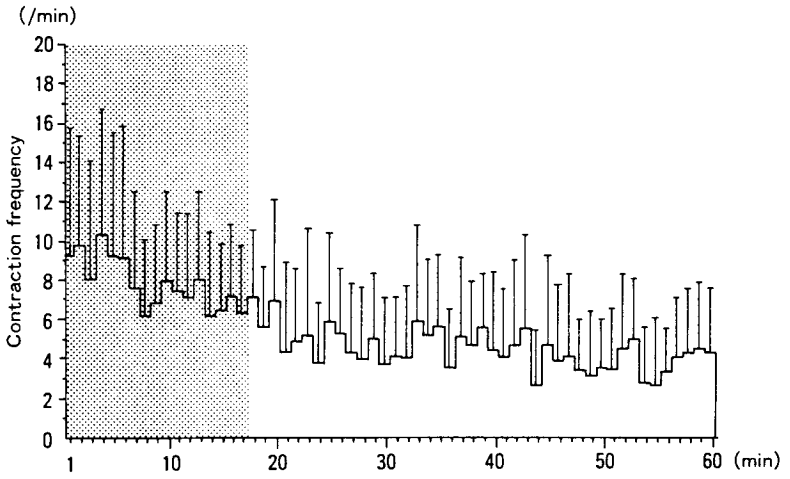

Fig. 1. Time course of lymphatic vasomotion after anesthesia and operative manipulation prior to hemorrhage. The mesenteric lymphatic contraction frequencies were unstable during the initial 17 min but stabilized after 18 min (58 min after anesthetic injection), and remained stable for 43 min (n=8).

groups, respectively. These differences were significantly different from pre-hemorrhage ($p<0.01$). Eighteen to twenty min post-hemorrhage (H-2), three groups (0.5ml/100g BW, n=5; 2ml/100g, n=6; and 2.5ml/100g, n=4) continued to show significant differences in contraction frequencies (70±17.2, 46±36.8, and 34±41.3%, respectively). The difference of the lymphatic contraction frequencies between the 0.5ml/100g and 2.5ml/100g hemorrhagic groups was statistically significant immediate post-hemorrhage (H-1) ($p<0.01$). Moreover, decreases in the lymphatic contraction frequency following hemorrhage were proportional to the volume of blood loss *(Fig. 2)*.

The absolute value of the relaxed diameter in the pre-hemorrhagic period for the four groups were 72±16.0μm and these did not change significantly at any time in each of the four groups prior to hemorrhage. The absolute value of the contracted diameter before hemorrhage for the four groups was 48±15.1μm and was reduced to 77±9.6 and 61±9.0% in the 1ml/100g and 2.5ml/100g hemorrhagic groups, respectively, immediately after hemorrhage (H-1) ($p<0.01$). The difference in contracted diameter in each of the four groups in the immediate post-hemorrhagic period (H-1) was also statistically significant ($p<0.01$). These decreases in the contracted diameter immediate post-hemorrhage (H-1) correlated with the volume of blood loss.

Retransfusion

In the immediate post-retransfusion period (R-1), lymphatic contraction frequency and contracted diameter recovered to pre-hemorrhagic values in each of the four groups. The relaxed diameters showed no change in the immediate and delayed post-retransfusion periods (R-1 and R-2). In the "delayed" post-retransfusion period (R-2), of the 2ml/100g group (n=6), however, lymphatic contraction frequency was still reduced to 42±30.3% compared with pre-hemorrhage ($p<0.01$). In the delayed post-retransfusion period (R-2), the lymphatic contracted diameter was within pre-hemorrhagic value for each group, but in the retransfusion period (R-1 and R-2), one rat in the 2ml/100g group and two rats in the 2.5ml/100g group had to be excluded because of lack of lymphatic contractions.

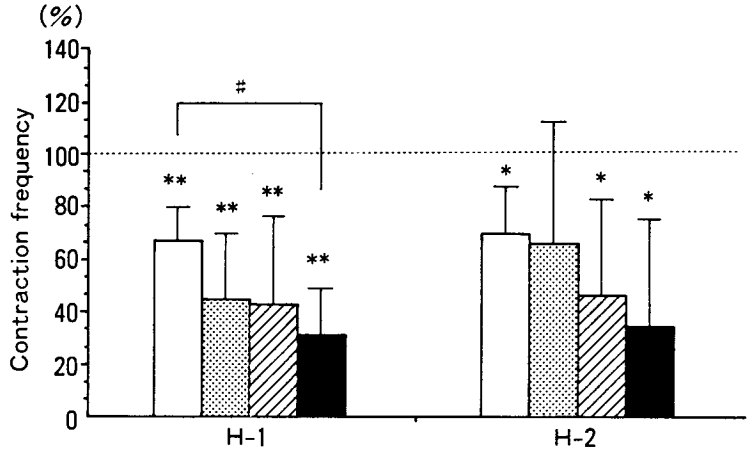

*Fig. 2. Changes in contraction frequency of rat mesenteric lymphatics (percentage of the pre-hemorrhage values). Data were obtained immediate post-hemorrhage (H-1) and 18 to 20 min after hemorrhage (H-2). In H-1, the contraction frequencies were decreased to 67±12.5, 45±24.7, 43±33.1, and 31±17.8% in each group (n=6). In H-2, lymphatic contraction frequencies were decreased to 70±17.2, 46±36.8, and 34±41.3% in the 0.5ml/100g (n=5), 2ml/100g (n=6), and 2.5ml/100g (n=4) groups, respectively. □:0.5ml/100g, square box with dots:1ml/100g, square box with slashes:2ml/100g, ■:2.5ml/100g. *p<0.05; **p<0.01 compared with pre-hemorrhage (students paired t test); #p<0.01 (ANOVA).*

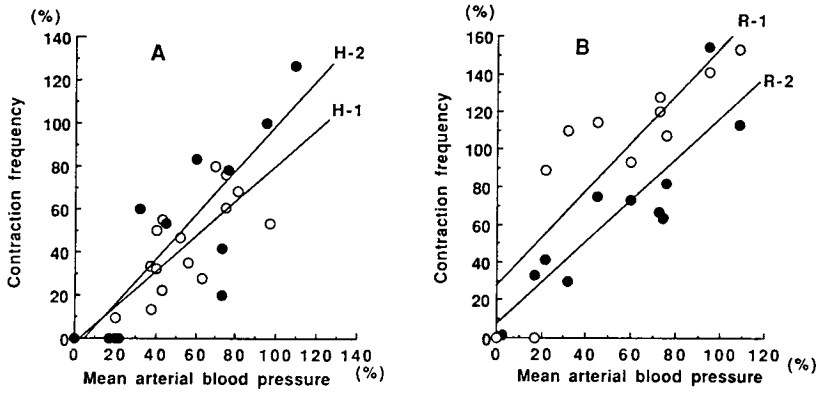

Fig. 3. (A) Relationship between the contraction frequency of mesenteric lymphatic vessels (expressed as percentage of the pre-hemorrhage) and the mean arterial pressure (expressed as percentage of pre-hemorrhage) in the immediate (H-1; O) and delayed (H-2; ●) post-hemorrhage period. In H-1 (n=16), correlation by regression analysis was R=0.76; p<0.01 and after H-2 (n=11), R=0.82; p<0.01. (B) Relationship between lymphatic contraction frequency immediately (R-1; O) and delayed (R-2; ●) post-transfusion and the mean arterial pressure 20 min after hemorrhage (H-2). The lymphatic contraction frequency in R-1 (n=11) and R-2 (n=11) correlated with the H-2 arterial pressure (in R-1, r=0.84, p<0.01; and in R-2, r=0.89, p<0.01).

Relationship between arterial pressure and lymphatic contraction frequency

The close association between lymphatic contraction frequency and mean arterial pressure in the post-hemorrhagic period are shown in *Fig. 3A* ["immediate" (H-1)—open circles and "delayed" post-hemorrhage (H-2)—closed circles]. The close association between the contraction frequency in the immediate (R-1) and in delayed post-transfusion periods (R-2) and the mean arterial pressure in the delayed post-hemorrhagic period (H-2) are shown in *Fig. 3B*.

DISCUSSION

Lymph propulsion arises from "active" and "passive" lymphatic driving forces. Passive forces include lymphatic compression and "suction" by extrinsic factors (7,8), whereas active factors derive from intrinsic contractions of lymphatic smooth muscle itself (8-10). The rate of spontaneous contractions evoked by distension of the lymphatic walls increases with a rise in intraluminal pressure (11-13). The lymphatic smooth muscle seems to play a pivotal role in the elastic behavior of the lymphatic wall and in the regulation of intrinsic contractility. These mechanisms are likely disrupted during hemorrhagic hypotension. Thus, before induction of blood loss there was minimal effect from anesthesia on mesenteric lymphatic vasomotion over 60 min and the instability observed during the initial 18 min probably resulted from the effect of operative manipulation. Based on these preliminary data, we were able to plan the appropriate experiments with hemorrhage and retransfusion.

According to Cope and Litwin (14), the flow of thoracic duct lymph increases sharply albeit transiently following a severe, but non-lethal, hemorrhage. Thereafter, lymph flow returns to a rate slightly below that before hemorrhage. After retransfusion, the flow of lymph in the thoracic duct increased in most, but not all, their animals (14). Johnston noted the effects on fluid propulsion and lymph flow were variable; nonetheless, there was a tendency for both pumping activity and lymph flow to be depressed following hemorrhage (15).

In our study, mesenteric lymphatic contraction frequency and contracted diameter were decreased following hemorrhage, and were proportional to the volume of blood removed. Most likely, net capillary filtration and therefore mesenteric lymph flow were decreased with hemorrhage. Hemorrhagic hypotension stimulates the baroreceptors and release of norepinephrine from sympathetic postganglionic nerve endings (16-18), which, in turn, produce constriction of the capillary beds. Moreover, plasma colloidal osmotic pressure within the microvasculature becomes relatively greater than the fall in capillary hydrostatic pressure thereby favoring return of tissue fluid directly into the blood circulation via the capillary membrane (19). Thus, there is less tissue fluid available to enter lymphatics and lymph flow therefore decreases (20-22). Another possibility to account for decreased lymph formation is with reduced blood flow to the intestinal villi during hemorrhage, pumping action of these villi is impeded and mesenteric lymph formation decreases with diminution in villous action (23).

In summary, mesenteric lymphatic contraction frequency and contracted lymphatic diameter decrease as lymph production falls with ongoing hypotension. This concept is favored by the close correlation between lymphatic contraction frequency and the level of arterial pressure. These data suggest that arterial pressure and by inference capillary blood flow and tissue oxygenation are important determinants of lymphatic contraction frequency.

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