Lymph Flow Dynamics into the Thoracic Duct of the Rat

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Summary Lymph flow into the thoracic duct of rats is reduced by 60% through immobilization with anesthesia. From this reduction, changes of the autonomic nervous activity by α -chloralose accounts for 20%. Pancuronium bromide blocking of the skeletal neuromuscular junction reduces lymph flow in the anesthetized rats by an additional 4%.

Key words: thoracic duct lymph, forces affecting lymph flow, rat.

Lymph flow into the thoracic duct is affected by many factors including lymph formation (YOFFEY and COURTICE, 1970), and anesthetics decrease lymph flow into the thoracic duct (SCHAD and BRECHTELSBAUER, 1977; YAMADA *et al.*, 1983). Some of the factors responsible for the decreased lymph flow into the thoracic duct during anesthesia are changes in the regional blood flow (MILLER *et al.*, 1980) and in the lymph formation in the regional organs (YAMADA *et al.*, 1983).

Immobilization (loss of skeletal muscle movement by anesthetics) of the animal is one of the factors that may decrease lymph flow into the thoracic duct. The lymph flow into the thoracic duct was also affected by other agents (BARROWMAN, 1978; OHHASHI *et al.*, 1978). No reports have been written on the quantitative measurement of the factors affecting lymph flow into the thoracic duct. In this study, thoracic duct lymph flow was examined both in awake rats and rats anesthetized with a variety of agents to demonstrate the effect both of the loss of skeletal muscle movement by anesthesia and of the autonomic nervous activity on lymph flow. Finally, the effect of pancuronium bromide blocking of the skeletal neuromuscular junction was studied.

Surgical procedure. Wistar strain rats weighing 200–600 g ($N=60$) were used. Rats were starved for 24 h but given water ad libitum. Surgical procedures were the same as reported previously (YAMADA *et al.*, 1983, 1984). In brief, the abdomen in the anesthetized rat was opened through a transverse left subcostal incision. After splenectomy, visceral organs were retracted with moist gauze to create the

operative field. The exposed thoracic duct behind the abdominal aorta was cannulated with a silicon tube (SH-OO, Dow Corning, 1.0 mm o.d.) below the diaphragm. The tube was exteriorized through the main incision. A tracheal catheter was used to avoid respiratory obstruction during anesthesia.

Awake rats were restricted in a modified Bollman cage, and given water *ad libitum* for 1 day of recovery after surgery.

Measurement. Lymph flow ($\mu\text{l}/\text{min}$ 100 g of body weight) into the thoracic duct was determined every 15 min for 6 h by the collection of lymph in a microtest tube (Eppendorf, Brinkman, Westbury, NY) and by measuring the weight of the microtest tube. The results which were obtained between 1–5 h were used for the analysis. Protein concentration of lymph was measured with a refractometer calibrated by the micro Kjeldahl method (YAMADA *et al.*, 1983). The amount of protein transported into the thoracic duct was calculated from the product of lymph flow and lymph protein concentration.

General protocol. Several anesthetic agents were used. α -Chloralose (10 mg/100 g of body weight) was administered intraperitoneally. Sodium thiopental (5 mg/100 g of body weight) was initially injected intraperitoneally, with anesthesia maintained by intravenous injection (1.5 mg/100 g of body weight) every 1 h. Urethane (0.25 g/100 g of body weight) was injected subcutaneously. Two % halothane in air was used to maintain anesthesia with a respirator (OS-R, Otani Co., Kyoto) using a constant volume (5 ml/stroke) at 60 times/min. Pancuronium bromide (5 μg /100 g of body weight, TAYLOR, 1985) was injected intravenously every 30 min to block the skeletal neuromuscular junction, and breathing was maintained by a respirator.

Statistics. The results were analyzed with the Student *t*-test and the null hypothesis was rejected when $p < 0.05$.

Table 1. Comparison of lymph flow, lymph protein concentration, and protein amount transported into the thoracic duct under various conditions.

	<i>n</i>	Lymph flow ($\mu\text{l}/\text{min}$ 100 g b.w.)	Lymph protein concentration ($\mu\text{g}/\mu\text{l}$)	Lymph protein amount into the thoracic duct ($\mu\text{g}/\text{min}$ 100 g b.w.)
Awake	47	$4.0 \pm 2.0^*$	29.4 ± 4.9	$114.5 \pm 51.3^*$
Chloralose	36	$2.4 \pm 1.2^{**}$	24.3 ± 6.4	53.1 ± 16.7
Thiopental	18	1.7 ± 0.4	31.2 ± 3.4	51.4 ± 21.6
Ether	26	1.8 ± 0.5	30.6 ± 3.4	53.3 ± 17.1
Urethane	17	1.5 ± 0.5	31.1 ± 2.9	44.7 ± 14.6
Halothane	21	1.5 ± 0.2	28.0 ± 2.2	40.4 ± 7.2

Values are mean $\pm$ S.D. * Means that the value is significantly ($p < 0.01$) higher than those of other groups. ** Means that the value is significantly ($p < 0.02$) higher than those under other anesthetic conditions.

Table 2. Effect of blocking of skeletal neuromuscular junction by pancuronium bromide on lymph flow, lymph protein concentration, and protein amount transported into the thoracic duct under anesthetic conditions.

	P.B.	n	Lymph flow ($\mu\text{l}/\text{min}$ 100 g b.w.)	Lymph protein concentration ($\mu\text{g}/\mu\text{l}$)	Lymph protein amount into the thoracic duct ($\mu\text{g}/\text{min}$ 100 g b.w.)
Ether	—	34	1.8 ± 0.6	30.9 ± 2.1	55.4 ± 17.3
	+	17	1.5 ± 0.7	29.1 ± 3.4	$43.3 \pm 19.2^*$
Thiopental	—	18	1.7 ± 0.7	32.5 ± 3.6	51.8 ± 25.4
	+	12	1.4 ± 0.7	28.2 ± 3.5	$36.1 \pm 13.5^*$

Values are mean $\pm$ S.D.; P.B., pancuronium bromide. * Means that the value is significantly ($p < 0.05$) lower than that under the control anesthetic conditions.

Lymph flow in awake rats varied widely, as can be seen in the standard deviations in Table 1. This is attributed to differences in muscular movement among animals (YAMADA *et al.*, 1983). Lymph protein concentration and the protein amount transported into the thoracic duct that were observed under the various conditions are also summarized in Table 1. The mean value of lymph flow in the awake rats was $4.0 \pm 2.0 \mu\text{l}/\text{min}$ of 100 g of body weight, and was significantly ($p < 0.01$) reduced to 40% by anesthesia ($1.6 \pm 0.6 \mu\text{l}/\text{min}$ of 100 g of body weight). Lymph flow into the thoracic duct in the group anesthetized by α -chloralose ($2.4 \pm 1.2 \mu\text{l}/\text{min}$ of 100 g of body weight) was reduced to 60%, which was higher ($p < 0.02$) than the average flow under anesthesia produced by other anesthetic agents. No significant difference in lymph flow was found among the other anesthetics. Lymph flow into the thoracic duct in animals in which the skeletal neuromuscular junction was blocked by pancuronium bromide under either ether ($1.5 \pm 0.7 \mu\text{l}/\text{min}$ 100 g of body weight) or thiopental anesthesia ($1.4 \pm 0.7 \mu\text{l}/\text{min}$ 100 g of body weight) was reduced 17 and 19%, respectively, more than the lymph flow reduction in the animals anesthetized without pancuronium bromide blocking (1.8 ± 0.6 and $1.7 \pm 0.7 \mu\text{l}/\text{min}$ 100 g of body weight, respectively) (Table 2).

Lymph protein concentration in the awake rats was $29.4 \pm 4.9 \mu\text{g}/\mu\text{l}$. Lymph protein concentration under anesthetic conditions was slightly increased, but not statistically significant. Lymph protein concentration in the rats anesthetized by α -chloralose was less ($24.3 \pm 6.4 \mu\text{g}/\mu\text{l}$) than in animals anesthetized with other agents.

The protein amount transported into the thoracic duct under the awake conditions ($114.5 \pm 51.3 \mu\text{g}/\text{min}$ 100 g of body weight) was significantly higher ($p < 0.01$) than the level in anesthetized animals. No significant difference in the amount of protein transported into the thoracic duct was found among anesthetized animals. In the chloralose-anesthetized group, the higher lymph flow and the low concentration of lymph protein yielded a similar amount of protein flow as in

animals anesthetized with other agents.

Immobilization by anesthesia reduced lymph flow into the thoracic duct by 60%: the contribution of the autonomic nervous system activity to lymph flow measured under chloralose anesthesia can be estimated as 18%. The additional 4% decrease in the lymph flow observed by blocking the skeletal neuromuscular junction demonstrates that the muscle pump is not completely blocked during anesthesia and that the muscle pump is a significant contributor to lymph flow because of the reduction of lymph flow by immobilization. Skeletal muscle activity in awake animals is the most significant factor determined lymph flow into the thoracic duct.

Significant increase in lymph flow and decrease in lymph protein concentration in animals anesthetized with chloralose, when compared to the results with other anesthetic agents, are in accord with earlier findings that lymph flow from the muscle is increased while lymph flow from visceral organs is decreased (YAMADA *et al.*, 1983). As α -chloralose increases the renal sympathetic activity and does not alter the blood pressure (SCHAD and SELLER, 1975), a redistribution of blood flow occurs: renal flow decreases and iliac flow increases (MANCIA and ZANCHETTI, 1981). Thus, the significant differences in lymph flow and lymph protein concentration between chloralose and other anesthetic agents can be attributed to the altered blood flow distribution mediated by the autonomic nervous system.

The protein amount transported into the thoracic duct depends almost entirely on the rate of lymph flow. Differences in lymph protein concentration under various conditions, although in some instances of statistical significance, are small. Therefore, the protein transported from the extravascular space to the vascular space through the thoracic duct is also primarily a function of the skeletal muscle activity.

In summary, lymph flow into the thoracic duct is affected by many factors. Immobilization by anesthesia reduces lymph flow by 60%, activation of the autonomic nervous systems by α -chloralose increases lymph flow by 20%, and blocking of the neuromuscular junction reduces lymph flow by an additional 4%.

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