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Neurogenic Influence on Pulmonary Surface Tension and Cholesterol in Cats[1] (38609)

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Previous clinical studies (1–4) have shown that the sympathetic nervous system plays a major role in the development of acute pulmonary injury which is associated with mechanical head injury, surgical trauma and severe nonspecific stress. Similar findings in animals exposed to experimental head injury (5–7), hyperbaric oxygen (8, 9), and sympathetic nerve stimulation (5, 10) suggest that, in addition to possible hemodynamic changes, sympathetic stimulation may alter the alveolar surfactants prior to the subsequent development of pulmonary congestion and edema (11). Previous work has shown that stimulation of the pulmonary sympathetics did not increase lung weight but decreased compliance and increased the minimum surface tension of the lung wash fluid as measured at maximal film compression on a surface tension balance (12, 13). Such changes occurred in animals under Na-pentobarbital anesthesia as well as ketamine. In view of a report (14) that the minimum surface tension of normal lung wash fluid is increased to abnormal levels (greater than 16 dynes/cm) by the addition of small amounts of cholesterol, the present experiments were carried out in order to determine what effect sympathetic nerve stimulation in cats might have on the cholesterol content of the alveoli.

Methods. Twenty-six adult cats (2–5 kg) were anesthetized with ketamine (35 mg/kg, im), the trachea cannulated, and the left stellate ganglion isolated via a dorsal retropleural approach, while avoiding rupture of the pleura. Both femoral arteries were catheterized and blood pressure recorded via a Statham P23AC transducer. Silver wire electrodes were placed on the ganglion and stimulation applied continuously for 5 minutes by a Grass S-9 stimulator (10 V, 0.5 msec duration, a frequency of 10/sec, square wave). Stimulation was considered effective if the systemic blood pressure rose 5–10 mmHg with a constant or slightly increased heart rate. Immediately following stimulation all animals were injected with heparin (Nutritional Biochem. Corp.) (12 mg/kg, iv) and rapidly exsanguinated via the femoral arteries. Upon cessation of ventricular contractions as determined by auscultation, the lungs were rinsed with 40 ml saline (0.9% NaCl). After a series of 10 alternate fillings and withdrawals with a syringe, all of the wash fluid that could be easily removed (about 30 ml of the original 40) was analyzed for cholesterol content by the Liebermann–Burchard color reaction (15).

Group I animals consisted of five cats exposed to electrical stimulation of the stellate ganglion and five sham-operated control animals treated identically except for application of the electrical stimulus. In a separate series of experiments (Group II) performed on eight additional cats for lung weight determinations, the saline rinse was omitted. The lungs were excised from these exsanguinated animals following stimulation or sham-operation, weighed, dried at 76° for 24 hr and weighed again for determination of lung wet wt/dry wt ratios and lung wt/body wt ratios.

In yet another series of experiments (Group III) the surface tension of the crude lung wash fluid was measured on a surface balance (Kimray Surfactometer) after 45 min of cycling from four cats exposed to this stellate ganglion stimulation, four sham-operated controls and also after the addition

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of 0.04 mg cholesterol dissolved in 1 ml petroleum ether to two control lung washes.

Results. The results from the present study show that sympathetic nerve stimulation via the stellate ganglion results in a nearly 200% increase in cholesterol in the lung wash fluid. This increase occurred in the absence of any evidence of gross lung pathology or lung weight increase. The cholesterol content of the lung wash fluid from the five stimulated animals (Group I) was 7.0 ± 1.6 (SE) mg compared to 2.3 ± 0.4 for five identically treated sham-operated controls ($P < 0.001$). In Group II animals the experimental lung weights were the same as were the controls. The mean lung wt/body wt ratio for four stimulated cats was 0.006, and for four controls 0.006. The mean lung wet wt/dry wt ratio for the four stimulated animals was 3.88 and for the four controls was also 3.88. In Group III the minimum surface tension measured on four cats exposed to stellate ganglion stimulation was 20.0 ± 1.3 dynes/cm compared to 4.5 ± 1.3 for four sham-operated controls ($P < 0.001$). The addition of 0.04 mg cholesterol to normal lung wash raised the minimum surface tension of two controls from 4.5 to 18.5 dynes/cm. Mean systemic blood pressure increased only 10–20 mmHg over the prestimulation level of 200 mmHg. Pulmonary artery pressure measured in four catheterized cats increased 1–2 mmHg during stellate ganglion stimulation. The lung wash fluid color, consistency, volume recovered and gross lung appearance were not affected by this stimulation.

Discussion. Previous work showed that sympathetic stimulation via the stellate ganglion in monkeys under Na-pentobarbital or ketamine anesthesia was associated with a decreased lung compliance and abnormally high minimum surface tension (5, 12). The results from the present study suggest the likelihood that an increase in intra-alveolar cholesterol may account for this high surface tension. It has been reported (14) that the addition of as little as 0.04 mg of cholesterol to the lung wash fluid of a rabbit increases the minimum surface tension to an abnormal level. In the present study the lung wash fluid of cats exposed to stellate ganglion stimulation contained an average of 4.7 mg more cholesterol than did controls.

The absence of any increase in lung weight reported in the present study following this stellate ganglion stimulation in spite of increased intra-alveolar cholesterol is not readily explainable except by assuming that any intraalveolar fluid which develops is either reabsorbed or else compensated for by a decrease in pulmonary blood volume. A decrease in blood volume might be expected (17) but would not likely significantly alter the lung weight of an exsanguinated animal. The reabsorption of alveolar edema fluid appears to be a more likely explanation for the normal lung weights since the development of pulmonary edema during stimulation of the sympathetic nervous system is well established (3). It was previously reported that this stellate ganglion stimulation increased intra-alveolar protein (16). It is not, however, expected that the presence of protein would alter the minimum surface tension (18), although it might be expected to extend the range over which a minimum value is obtained.

Previous findings showed that a decreased lung compliance and an abnormally high minimum surface tension occurred immediately following mechanical head injury in the absence of gross lung pathology (5). These compliance decreases were blocked by various sympatholytic agents and were thus attributed to a sympathetic action (5), as were surfactant changes found after stress from exposure to hyperbaric oxygen (9). Previous work also showed that sympathetic nerve stimulation via the stellate ganglion in monkeys resulted in a decreased compliance and a high minimum surface tension (12). It has been reported (14) that the addition of small amounts of cholesterol to lung wash fluid markedly raises the minimum surface tension. In the present study large increases in cholesterol were found in the lung wash fluid following this stellate ganglion stimulation. Thus the results from the present study suggest that the decrease in compliance reported previously and the high minimum surface tension associated with sympathetic stimulation in monkeys and cats may be due at least in part to an increase in intra-alveolar cholesterol.

Summary. Previous work showed that stress involving the sympathetic nervous

system via mechanical head injury and hyperbaric oxygen results in a decreased lung compliance and altered alveolar surfactants. Similar changes were associated with sympathetic nerve stimulation via the stellate ganglion. In view of reports that the minimum surface tension attained by lung wash fluid is increased by very small amounts of cholesterol, the present experiments were performed in order to determine what effect sympathetic stimulation in the cat might have on the cholesterol content of the alveoli. The results show a nearly 200% increase in intra-alveolar cholesterol as well as high minimum surface tensions following sympathetic nerve stimulation. Such changes developed in the absence of any increase in lung wet wt/dry wt ratios. The results from the present study suggest that the previously reported decreased lung compliance and increased minimum surface tension associated with sympathetic stimulation may be due at least in part to contamination of the alveolar surfactants with large amounts of cholesterol.

1. Ducker, T. B., J. Neurosurg. **28,** 112 (1968).
2. Hess, L., Wein Med. Wochschr. **84,** 285 (1934).
3. Visscher, M., Haddy, F. J., and Stephens, G., Pharmacol. Rev. **8,** 389 (1956).
4. Weisman, S., Surgery. **6,** 722 (1939).
5. Beckman, D. L., Bean, J. W., and Baslock, D. R., J. Appl. Physiol. **30,** 394 (1971).
6. Ducker, T. B., and Simmons, R. L., J. Neurosurg. **28,** 118 (1968).
7. Eichbaum, F. W., Gazetta, B., Bissetti, P. C., and Pereira, C. B., Z. Ges. Exp. Med. **139,** 721 (1965).
8. Bean, J. W., Physiol. Rev. **25,** 1 (1945).
9. Beckman, D. L., and Houlihan, R. T., Aerospace Med. **44,** 422 (1973).
10. Beckman, D. L., and Mason, K. F., Life Sci. **12,** 43 (1973).
11. Beckman, D. L., and Bean, J. W., Chest. Suppl. **65,** 38 (1974).
12. Beckman, D. L., Bean, J. W., and Baslock, D. R., J. Trauma. **14,** 111 (1974).
13. Droste, P. L., and Beckman, D. L., Proc. Soc. Exp. Biol. Med. **146,** 352 (1974).
14. Tierney, D. F., and Johnson, R. P., J. Appl. Physiol. **20,** 1253 (1965).
15. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., J. Biol. Chem. **195,** 357 (1952).
16. Beckman, D. L., Sexton, J. D., and Bergren, D. R., Fed. Proc. **33,** 346 (1974).
17. Szidon, J. P., and Fishman, A. P., Amer. J. Physiol. **220,** 364 (1971).
18. Hurst, D. J., Kilburn, K. H., and Lynn, W. S., Resp. Physiol. **17,** 72 (1973).

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