Effects of Epsilon Amino Caproic Acid on Pulmonary Vascular Changes Produced by Snake Venom.* (28507)

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Snake venoms are complex substances which produce a variety of abnormalities in mammals. Despite considerable study of the action of venoms over a period of many years, the mechanism of their lethal effects remains poorly understood. In the experiments to be reported below, some of the vascular changes produced in the isolated canine lung by venoms of *Naja hannah* and *Crotalus adamanteus* have been studied. Results indicate that these venoms affect the vascular wall through the medium of a heat labile non-cellular component of blood.

Material and method. Isolated, freshly excised canine lungs were suspended from a strain gauge balance and perfused at constant flow utilizing a Sigma-Motor pump. Perfusion pressure and weight change were recorded by a Sanborn recorder while the lung was aerated with room air by means of a mechanical respirator. The open pulmonary veins drained into a funnel-shaped reservoir beneath the scale pan which returned the blood to the pump-oxygenator system. Pressure in large veins was thereby maintained at zero level during perfusion. Pulmonary artery flow rates varied from 220 ml/min to 380 ml/min in different experiments but flow was maintained constant throughout any given experiment. All preparations were perfused for 30 minutes prior to introduction of 10.0 mg venom of *C. adamanteus* or 10.0 mg venom of *N. hannah* into the perfusing fluid. Dried venom (obtained from Ross Allen Reptile Institute) was reconstituted with normal saline (1 mg/1 ml) and rapidly injected *via* a syringe and 18-gauge needle directly into the tubing of the pump system. In this manner minimum dilution of the venom occurred prior to its entry into the lung. The total volume of the perfusate was 300 to 500 ml. Canine blood or blood derivatives were utilized except in experiments requiring dextran (Gentran, mol wt 75,000) for perfusion. The presence of pulmonary edema was judged by weight gain of lung during perfusion and was confirmed by microscopic examination of hematoxylin and eosin stained sections of all lungs studied. When intravascular thrombosis occurred, it was apparent on examination of blood in the clear plastic tubing during perfusion. All lungs were microscopically examined for intravascular thrombi as well. The following series of experiments were carried out using each of the 2 venoms, *N. hannah* and *C. adamanteus*:

1. Effect of venoms on isolated lung.

a. *Isolated lung, venom.* After perfusion for 30 minutes, 10.0 mg of either venom was added to the perfusing medium and changes in pressure or weight recorded. Twenty-four experiments were carried out with each venom. Lungs were perfused utilizing whole blood (8 experiments), plasma prepared from heparinized† whole blood (4 experiments), serum removed after allowing fresh canine blood to clot (4 experiments), heated plasma prepared by warming to 56° C for 30 minutes (3 experiments), whipped or defibrinated blood (2 experiments), dextran (3 experiments).

b. *Isolated lung, 48/80 and venom.* In 4 experiments with each venom, the lung was perfused with whole blood for 30 minutes. Five mg of 48/80, a substance known to release endogenous histamine(1) was added to the perfusing fluid and 20 minutes thereafter 10.0 mg venom was added.

c. *Isolated lung, histamine, antihistamine, venom.* In 4 experiments using each venom, the following sequence was carried out. The lung was perfused with whole blood for 20

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† Heparin, Sodium: Upjohn; 50 U.S.P. units per cc of blood.

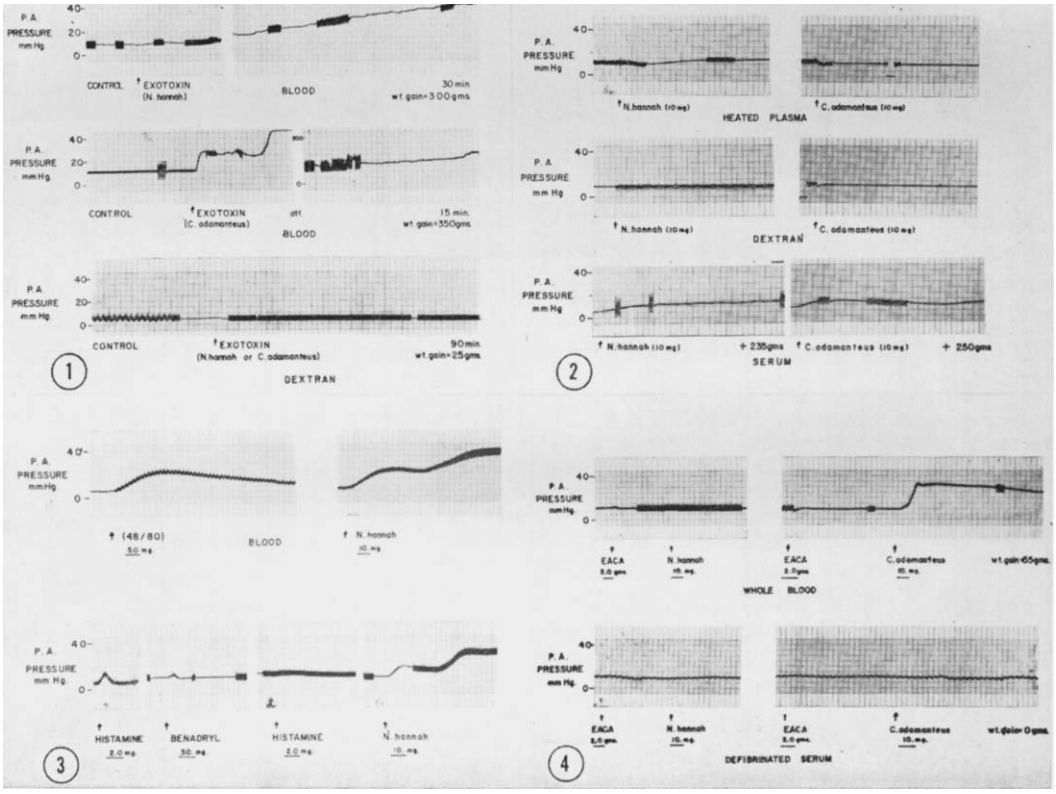

FIG. 1. Response of isolated, perfused lung to: (a) Upper tracing: *N. hannah* in whole blood. (b) Middle tracing: *C. adamanteus* in whole blood. (c) Lower tracing: *N. hannah* or *C. adamanteus* in dextran (mol wt 75,000). Time and weight gain indicated in each case.

FIG. 2. Response of isolated lung to *N. hannah* and *C. adamanteus* when perfused with heated plasma, dextran, or serum.

FIG. 3. Response of isolated lung perfused with whole blood. (a) Upper tracing: comparison of effect of 48/80 with *N. hannah*. (b) Lower tracing: effect of histamine, before and after antihistamine, and effect of *N. hannah* after blocking of histamine effect.

FIG. 4. Effect of *N. hannah* and *C. adamanteus* following EACA in isolated lung perfused with: (a) Upper tracing: whole blood. (b) Lower tracing: defibrinated blood.

minutes. Two mg of histamine was injected followed by 50 mg of an antihistamine, Benadryl. A second test dose of 2 mg of histamine was then given, after which 10 mg of venom was injected.

d. *Isolated lung, EACA,‡ venom.* In 6 perfused lungs, EACA (2 g/8 ml normal saline) was added to the perfusing medium just prior to addition of 10.0 mg of Cobra venom. In 3 of these experiments heparinized whole blood and in 3 others defibrinated blood was utilized for perfusion. The experiments were repeated with 6 additional lungs using rattlesnake venom.

‡ Epsilon Amino Caproic Acid. Kindly supplied by Lederle Laboratories, Pearl River, N. Y.

Results. Effects of N. hannah *venom in the isolated lung.* The results of introduction of venom into the isolated perfused canine lung are shown in Fig. 1 (upper tracing). There is immediate increase in perfusion pressure, (pulmonary artery pressure) increase in weight and obvious pulmonary edema. If the lung is perfused with oxygenated plasma, serum, or defibrinated blood, the response is identical to that observed with whole blood. However, if plasma is heated to 56° C for 30 minutes prior to perfusion, venom fails to evoke the characteristic response (Fig. 2). This negative response to venom is also noted in lungs perfused with dextran (Fig. 1, 2). In lungs perfused with whole blood, 48/80

produces a rise in pulmonary artery pressure but little or no associated edema. After approximately 10 minutes the lung appears normal and pulmonary artery pressure returns to baseline values. *N. hannah* venom introduced thereafter produces the characteristic response (Fig. 3). In blood perfused lung, histamine produces little or no effect when compared with venom (Fig. 3). In addition, Benadryl, which blocks the action of histamine in the lung tissue, does not alter the characteristic response of the same lung to venom (Fig. 3).

Epsilon amino caproic acid (EACA) introduced into the blood prior to venom inhibits the response to venom. Lungs were perfused for 90 minutes following venom without the occurrence of significant weight gain, edema, or gross and microscopic changes (Fig. 4). In defibrinated whole blood or serum, EACA likewise completely inhibits the effect of *N. hannah* venom on the lung (Fig. 4).

Effect of C. adamanteus *venom in isolated lung.* The initial response to *C. adamanteus* venom in the isolated blood-perfused canine lung resembles that seen with *N. hannah* venom. The pulmonary artery pressure rises, weight increases and the lung becomes edematous. This is followed in a few minutes by a sharp secondary rise in pulmonary artery pressure and clots are visible in the external circuit of the preparation. It is usually impossible to perfuse the lung for more than a few minutes after intravascular coagulation and often the pulmonary artery ruptures at this point. Tracing from a typical experiment is seen in Fig. 1 (middle tracing). If plasma is utilized for perfusion, the response to venom is identical to that in blood. Perfusion using serum or defibrinated blood resulted in a response to *C. adamanteus* identical to that seen with *N. hannah.* With heated plasma or with dextran, venom causes no response in the lung (Fig. 2).

Following introduction of 48/80, pulmonary artery pressure rises but edema does not occur. Thereafter, *C. adamanteus* venom produces a typical response in the lung. Even following the blocking of a histamine response by an antihistamine (Benadryl) the venom retains its capability of producing a typical response in the lung.

Introduction of EACA into the blood prior to *C. adamanteus* venom completely inhibits the primary vascular response. Clotting occurs, however, and the pulmonary artery pressure rises sharply. Pulmonary edema does not occur. EACA inhibits the vascular response of this venom in lungs perfused with defibrinated whole blood or serum, and, as might be expected, no clotting occurs (Fig. 4).

Discussion. To produce the pulmonary edema and interstitial hemorrhage observed in the lung after administration of venoms a heat labile non-cellular component of blood must be present. The introduction of either venom into the isolated canine lung perfused with dextran or heated plasma failed to result in the characteristic edema and hemorrhage seen when the lung is perfused with blood, plasma or serum.

Administration of a compound known to release endogenous histamine from tissues (48/80) produces only transient increases in pulmonary artery pressure without significant gains in lung weight or pulmonary edema. Similarly, administration of histamine does not result in the characteristic changes seen with either venom. Further, venom of *N. hannah* or *C. adamanteus* introduced into the circulation of an isolated lung after these compounds still results in the characteristic changes of increased pressure, edema and hemorrhage. An anti-histaminic drug, Benadryl, which blocks the effects of histamine in the lung, does not prevent nor alter the characteristic responses to venom. On the basis of these experiments, we cannot agree that histamine plays an important role in the vascular effects of venom in lung(2,3,4). Feldberg and Kellaway noted, however, that the direct effects of cell injury must be considered as well as histamine release to explain pulmonary changes induced by copperhead (2) or cobra venom(3,4).

The effect of EACA, which completely prevents the edema and hemorrhage induced by either venom in the lung, suggests that this

substance may interfere with activation of an enzyme system. Although EACA is an inhibitor of plasminogen activation(5,6,7), it may have other actions as well(8,9). Edema and hemorrhage are not produced in isolated perfused canine lung by administration of fibrinolysin which suggests that some mechanism other than activation of fibrinolysin is involved in venom action(10). This and other experiments to clarify the mechanism of venom action are in progress. As expected, EACA has no demonstrable effect on the intravascular coagulation produced by rattlesnake venom.

Summary. 1. The pulmonary vascular effects of edema and hemorrhage produced by *C. adamanteus* and *N. hannah* venoms are mediated through a heat labile non-cellular component of blood. 2. Venom of *C. adamanteus* also produces intravascular thrombosis not seen with venom of *N. hannah*. 3. The vascular effects of both but not the coagulating property of *C. adamanteus* are completely prevented by Epsilon Amino Caproic Acid. 4. The best explanation is that this compound prevents activation of an enzyme system which is responsible for the vascular changes.

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Endocrine Stimulating Effects of Yohimbine-HCl. (28508)

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Many conflicting reports are found in literature concerning the effects of yohimbine on the reproductive cycle of rodents. Rumry(1) reported that 1 mg of yohimbine per animal per day for 8 months was without effect on the number of litters produced by white mice. Ludwig and Ries(2) showed 0.05 to 0.10 mg of yohimbine produced typical conditions of estrus in 6-8 g immature mice. D'Amour(3) demonstrated hyperemia of the genital organs of 21-day-old rats by daily injections of 0.4 mg of yohimbine per animal for 10 days, but was unable to produce vaginal opening. Four milligrams of yohimbine for 7 days did not produce cornified vaginal smears in ovariectomized rats. Fugo and Gross(4) reported that injections of 1 to 2 mg of yohimbine-HCl per animal per day cause the estrous cycles of the adult female rat to be delayed. Three milligrams per day produced a pseudopregnant condition and male-like behavior. Spayed or hypophysectomized female rats did not exhibit estrus after yohimbine treatment. Corpora lutea formed in ovaries transplanted to the eyes of male rats treated with yohimbine.

Sulman and Black(5) found daily injections of 1-4 mg of yohimbine-HCl for 5 weeks to be without effect on estrous cycles, ovarian weight, or body weight of 60- to 100-day-old female rats. They reported no effect with 0.3 to 3.0 mg of yohimbine in the Allen-Doisy test on spayed mice or infantile rats, no gonadotropic effects of 0.5 to 5.0 mg of yohimbine in infantile rats, and no difference in time of first estrus or in the periodicity of vaginal estrus with 0.1 to 3.0 mg of the drug.