Vasoconstrictive Action of Epinephrine Following Staphylococcal Enterotoxin.* (31161)

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Intravenous administration of staphylococcal enterotoxin, the causative agent of staphylococcus food poisoning(1), alters the skin of rabbits so that a hemorrhagic response develops in the area of a subsequent intradermal epinephrine injection. Enterotoxin and epinephrine injected into the same intradermal site elicit a similar lesion(2). This host response to staphylococcal enterotoxin is comparable to those caused by bacterial endotoxin(3) and represents one of the interesting similarities in the actions of these two different toxins(4).

The dermal response to epinephrine in animals treated with bacterial endotoxin (LPS) may result from an effect of this toxin on the reactivity of small blood vessels to epinephrine. Topical administration of amounts of epinephrine which normally have little or no effect provokes intense vasoconstriction in the meso-appendix microcirculation of rats treated with small doses of LPS. High doses of the toxin result in hyporeactivity of the circulation to epinephrine(5). The present study shows that staphylococcal enterotoxin possesses corresponding properties.

Materials and methods. Two serologically different enterotoxins(6) of 95% or more purity were dissolved in pyrogen-free saline for i.v. injections. Enterotoxin B was obtained by previously reported methods(7,8) while Enterotoxin A was purified by an unpublished procedure (M. S. Bergdoll, Food Research Inst.). These preparations originally provoked vomiting in about half of rhesus monkeys challenged *per os* at a level of 10 μg per animal, but ED_{50} of Enterotoxin B at the time of the present experiment was 30 μg. Lipopolysaccharide *Salmonella enteritidis* endotoxin (Difco Laboratories, control No. 128076) was dissolved in pyrogen-free saline by vigorous shaking after heating in an 80°C water bath for 15 min.

The vascular reactivity to epinephrine was studied by adaptation of the rat meso-appendix procedure(9). New Zealand male rabbits of 2 to 3 kg were anesthetized with sodium pentobarbital and that portion of the intestinal tract necessary to spread out the mesentery between the ileum and appendix was exteriorized. The tissue was maintained at body temperature by irrigation with prewarmed Ringer-gelatin solution and the microcirculation observed at 60× magnification.

Two ml of 2-fold dilutions of epinephrine were dripped from a pipette over a 15-sec period onto the selected portion of the meso-appendix. The minimal epinephrine concentration which narrowed the precapillaries and stopped the blood flow in the capillaries was taken as the epinephrine threshold concentration (E.T.C.). Vasoconstriction usually appeared within 1 minute of epinephrine application; when no response occurred observation was continued for about 3 minutes. Unless the E.T.C. was greatly exceeded, washing with Ringer-gelatin restored the microcirculation so that the next drug concentration could be tested within 3 to 4 minutes.

The E.T.C. was determined at selected time intervals after i.v. toxin challenges. When toxin effects of 3 hours or less were to be studied, a pre-toxin E.T.C. was often determined on the same animal.

Results. E.T.C. for 35 normal rabbits was 0.375 $\pm$ 0.233 μg/ml (mean $\pm$ standard deviation). Effectiveness of epinephrine in individual rabbit retested several times over a 3-hour period was fairly constant. Usually the difference between the maximum and minimum E.T.C. for a given animal was 2-fold; a 4-fold spread was obtained in one animal.

The effects of staphylococcal enterotoxin on responsiveness to epinephrine are ex-

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TABLE I. Vasoconstrictive Effect of Epinephrine on Meso-Appendix Microcirculation of Rabbits Administered Staphylococcal Enterotoxin and Bacterial Endotoxin.*

Challenge (μg/kg)	Hours after challenge: ½	3	10	18	24
Saline	1.5 (5)	1.4 (4)	—	1.5 (2)	—
Enterotoxin B					
.2	1.0 (3)	7.5 (4)†	—	3.0 (3)	—
1.0	1.0 (4)	6.1 (5)†	—	8.4 (5)†	—
2.0	2.4 (7)	7.0 (6)†	15.0 (5)†	11.2 (4)†	11.2 (4)†
10.0	1.0 (6)	6.5 (4)†	27.0 (4)†	20.0 (3)†	—
100.0	.2 (5)†	.2 (6)†	—	—	—
Enterotoxin A					
2.0	—	10.5 (4)†	—	28.0 (4)†	—
Endotoxin					
10.0	—	31.5 (4)†	—	8.2 (4)†	—

* As average of ratios of mean E.T.C. for control animals to individual test animals; figures in parentheses = No. of animals.

† Significant at $P \leq .05$ when E.T.C. of controls compared to test group by the "t" test.

pressed as the average of ratios of mean E.T.C. for control animals to individual toxin-treated animals. The results are not strictly quantitative but indicate the general trend of the enterotoxin effect (Table I). Considerable variation in E.T.C. occurred in certain toxin-treated groups. However, if that group as a whole was significantly more sensitive to epinephrine than the control group, the individual E.T.C. was never larger than the average of the controls.

Topical application of staphylococcal enterotoxin to the meso-appendix for several hours had no significant effect on the E.T.C. The results with LPS were those expected from data obtained by other investigators(5, 10).

Without prior treatment with epinephrine, petechial hemorrhages were practically never observed in the meso-appendix of control rabbits. In contrast, some 25% of animals administered enterotoxin (2 to 10 μg/kg) 10 to 24 hours previously showed these lesions before the E.T.C. determination was started. Petechiae induced by epinephrine were much more extensive in the toxin-treated animals.

Discussion. Highly purified Enterotoxins A and B affect the responsiveness of the peripheral circulation of the rabbit mesentery to epinephrine. Increase in sensitivity to the vasoactive hormone develops slowly; maximum sensitization occurs about 10 hours following enterotoxin injection. Rapid loss of vasoconstrictive response to epinephrine follows an enterotoxin dose normally lethal to rabbits within 12-24 hours (100 μg/kg). The dose dependent, biphasic change in the responsiveness to epinephrine is similar to that following LPS administration to rats and rabbits. The LPS effect differs from that of enterotoxin in that the maximum hyper-reactive state develops earlier(5,10). Regardless of the difference in temporal sequences, adrenergic factors seem involved in this response to either of these toxins.

The contractile response to epinephrine of the spirally cut rabbit aorta preparation(11) bathed in Krebs-Ringer solution is not affected by LPS(10) or enterotoxin. The vascular smooth muscle exhibits greater reaction to epinephrine in the presence of LPS when the suspending solution is supplemented with blood(12) and the interaction of the toxin with blood cells may be the basis of the LPS-induced change in vasoconstriction of the meso-appendix to the hormone. Similar augmentation in epinephrine response is not obtained even when the aortic preparation is incubated up to 2 hours in blood-enterotoxin mixture. Thus, the similar action of the food poisoning toxin on the vasoreactivity of the rabbit meso-appendix to epinephrine probably involves an indirect mechanism(4).

Summary. The effects of staphylococcal enterotoxin on the sensitivity of the microvasculature of the rabbit meso-appendix to

epinephrine was studied. Intravenous injection of even 0.2 μg/kg of the food poisoning toxin induced a significant increase in the vasoconstrictive effect of topically applied epinephrine within 3 hours. Maximum sensitivity occurred about 10 hours after toxin administration and persisted over 18 hours with toxin levels of 1 to 10 μg/kg. A rapidly developing state of hyporeactivity to epinephrine resulted from much larger enterotoxin challenges. Except for the much slower evolution of the state of increased responsiveness to epinephrine, these results are similar to those induced by bacterial endotoxin.

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Density Gradient Centrifugation Studies of Rubella Virus. (31162)

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Little information is available on the physical and chemical characteristics of rubella virus. Norrby *et al*(1) reported that the virus could not be sedimented when placed on a gradient in which the lowest density was 1.08 g/cm^3. Others (Sever *et al*(2); Parkman *et al*(9); Cusumano *et al*(5)) have published data on the size, nucleic acid, and sensitivity to heat and chemical agents of this virus. A major impediment to more detailed physicochemical study of this virus has been the fact that rubella titers in tissue culture are only in the range of 2 to 4 Log_{10} $TCID_{50}$/0.2 ml, and rarely 5 Log_{10} $TCID_{50}$. The recent availability of techniques for rapidly concentrating large volumes of virus suspension has permitted the practical use of density gradient centrifugation in the study of this virus.

Materials and methods. Virus. Rubella virus was grown in each of 3 cell lines. Two strains of virus were used. The RV strain (Sever *et al*(2)) was isolated in this laboratory and was passed 14 times in African green monkey kidney (AGMK) cells. A subline of the RV strain rubella virus was propagated in RK-13 continuous rabbit kidney cells (McCarthy *et al*(8)). A third rubella virus preparation was obtained from a chronically infected strain of LLC-MK_2 continuous rhesus monkey kidney cells (Brown *et al*(3)).* It should be noted that all known rubella virus strains are serologically indistinguishable (Sever, J. L.—unpublished data).

Monolayers of each infected cell line were grown in 32 oz bottles according to previously described methods (Sever *et al*(2,4)); McCarthy *et al*(8)) and where necessary were inoculated with rubella virus. Fluids from infected and control cultures of each cell line were harvested three times a week during the life of the culture and were stored at −70°C until used. All virus assay procedures were conducted in AGMK tissue cultures as previously described (Sever *et al*

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