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## Failure to Observe Alteration of Epinephrine Activity after Endotoxin. (24602)

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(Introduced by Maurice B. Visscher)
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Zweifach, Nagler, and Thomas(1) have reported changes in epinephrine activity in blood vessels in the isolated rabbit ear and in mesoappendix of the rat following administration of endotoxin. In the rabbit ear they found that perfusion of 0.5 to 2 μg of endotoxin potentiated epinephrine reactivity and 40 to 50 μg caused reversal. Hinshaw, Gilbert, Kuida, and Visscher (to appear) were unable to demonstrate an altered vascular reactivity to epinephrine following injection of endotoxin in the isolated blood-perfused dog forelimb and lung. This prompted us to reinvestigate the responses in the isolated rabbit ear.

*Method.* The perfusion system was identical to that described(1), but in addition pressure recording equipment was used. The system consisted of a reservoir above a volumetric pipette calibrated in 0.1 cc. The ear was cannulated with a 22 gauge needle and connected to the pipette by a rubber tube. Tyrode solution was used as the perfusing fluid. Test doses in 0.1 cc were injected immediately upstream to the cannula. A needle in a polyethylene catheter was inserted into the rubber tubing at the level of cannulation, about 15 cm upstream, connected to a strain gauge, and the pressure recorded on a Sanborn twinviso recorder. The change in meniscus level was used to indicate alterations in resistance to flow through the ear. A rise in the meniscus level indicated increased resistance and increased perfusion pressure. Meniscus readings were taken at 1 minute intervals after epinephrine injections until perfusion pressure began to change. The meniscus was then readjusted to return the flow rate to the control value, *i.e.*, outflow from pipette was readjusted to constant inflow from the reservoir. It was required that the meniscus level remained stabilized for at least 1 minute before another injection of epinephrine. Lipopolysaccharide *E. coli* endotoxin from Difco Laboratories was used. The activity of this endotoxin was verified by assay in anesthetized dogs. Endotoxin and Tyrode perfusate were prepared with either laboratory distilled water for 3 experiments or in pyrogen-free water from Abbott Laboratories in the remaining experiments. No differences were noted.

*Results.* The epinephrine response after administration of a 0.5 μg endotoxin dose was studied 16 times in 12 ear preparations. Of these 12 preparations 4 ears were removed under ether anesthesia, 3 under nembutal, and 5 after decapitation. Flow rates varied from 2 to 3.4 cc/min. Absolute perfusion pressures were 37 to 92 cm $H_2O$ in different preparations. Control epinephrine doses were 0.01 μg in 1 experiment; 0.001 in 10 experiments; 0.0001 in 3 experiments; and 0.00001 in 2 experiments. In several experiments control studies were performed to evaluate the consistency of epinephrine response (Table I). Usually only 2 injections were made with the same epinephrine dose prior to administration of 0.5 μg of endotoxin to decrease the length of the experiment to avoid excessive edema.

Zweifach *et al.* administered 0.5 μg of epine-

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TABLE I. Control Epinephrine Responses in Isolated Rabbit Ear.

| Exp. | Change in meniscus level (mm) A. 0.01 μg | B. 0.001 μg |
|---|---|---|
| 2-32 | 11.5 | 3.0 |
| | 10.0 | 4.0 |
| | 11.5 | 2.5 |
| | 10.0 | 1.5 |
| 2-33 | 7.5 | .0 |
| | 7.0 | .0 |
| | 5.0 | .5 |
| | | .0 |
| 2-41 | 14.0 | 6.5 |
| | 9.0 | 3.0 |
| | 10.5 | 3.5 |
| | | 5.0 |

phrine as a priming dose to test reactivity of the ear. In their sample protocol this dose elicited a 5.5 mm rise in the meniscus level. In our first preparation this epinephrine dose was injected and it gave such a marked response (171 mm rise in the meniscus level) that the pipette overflowed, the preparation never returned to control flow values and had to be discarded. In another ear a similar response was observed (178 mm rise, and an overflow of the pipette) and 42 minutes elapsed before the ear returned to control flow.

Only in 2 out of 4 experiments in which the ears were removed under Nembutal anesthesia, a small potentiation was observed after injection of 0.5 micrograms of *E. coli* endotoxin (Table II). Zweifach *et al.* reported observing 200 to 850-fold potentiation in the 5 experiments for which they presented data.

In 9 of the 12 ear preparations 50 μg of *E. coli* endotoxin were given either before or after investigating epinephrine activity with the small dose of endotoxin. Flow rates varied from 2.0 to 3.1 cc/min. In this series of 10 experiments the control dose of epinephrine was usually larger than those in our previous investigation using the smaller endotoxin dose. The response in 7 of the 10 experiments to the same dose of epinephrine tended to be less than the control response. A drop in the meniscus level was not seen, that is, a reversal of response was never observed.

The results obtained from the pressure recording of one preparation using the 2 different endotoxin doses are shown in Fig. 1. The change in perfusion pressure in millimeters of water is equivalent to the change in meniscus level. Flow rates were the same for both experiments. It is apparent that the epinephrine responses were essentially unchanged after either 0.5 or 50 μg of endotoxin.

*Conclusions.* 1) The fact that potentiation of the action of epinephrine after small doses

TABLE II. Epinephrine Activity in Isolated Rabbit Ear Associated with Administration of 0.5 μg of *E. coil* Endotoxin.

| Exp. | Tests before endotoxin: Dose | Response, mm $H_2O$ | Dose | Response, mm $H_2O$ | Tests after endotoxin*: Dose | Response, mm $H_2O$ | Dose | Response, mm $H_2O$ | Dose | Response, mm $H_2O$ | Flow, cc/min. | Perfusion pressure, cm $H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 E | .001 | 1.5 | .0001 | .0 | .001 | 1.5 | .001 | 1.0 | .001 | 1.5 | 2.7 | 57 |
| 2 E | " | 3.0 | " | 1.5 | " | 1.0 | " | 1.0 | " | 3.0 | 3.1 | 47 |
| 3 E | " | 3.0 | | | " | 1.0 | " | 2.5 | " | 1.0 | 3.1 | 48 |
| 4 E | .01 | 25.5 | .001 | 7.0 | " | 5.5 | " | 7.5 | " | 7.5 | 3.0 | 68 |
| 5 E | " | 30.0 | " | 2.0 | " | 1.5 | " | 2.5 | " | 2.0 | 2.4 | 47 |
| 1 D | .001 | 2.0 | .0001 | .5 | .0001 | .5 | .0001 | .0 | .0001 | .5 | 3.1 | 45 |
| 2 D | " | 6.5 | " | 2.0 | .00001 | .0 | .00001 | .0 | | | 3.0 | 37 |
| 3 D | " | 2.0 | " | .5 | .0001 | .5 | .0001 | .5 | " | .0 | 3.1 | 47 |
| 4 D | " | 6.5 | | | .001 | 6.5 | .001 | 6.5 | .001 | 7.0 | 3.0 | 37 |
| 5 D | .0001 | 8.0 | .00001 | 3.0 | .00001 | 3.0 | .00001 | 3.0 | | | 2.7 | 39 |
| 6 D | " | 8.0 | " | 2.5 | " | 3.0 | .0001 | 8.5 | .0001 | 7.5 | 3.0 | 50 |
| 7 D | .001 | 1.5 | .001 | 1.0 | .001 | 2.0 | .001 | 1.0 | | | 2.9 | 34 |
| 1 N | .01 | 2.0 | " | .0 | .0001 | .0 | " | .5 | .001 | 1.0 | 3.4 | 46 |
| 2 N | .001 | 1.0 | .0001 | .0 | .001 | 1.5 | .0001 | .5 | .0001 | .0 | 3.3 | 51 |
| 3 N | " | 2.0 | " | .0 | .0001 | 2.0 | .001 | 2.0 | | | 2.0 | 92 |
| 4 N | " | 1.0 | " | .0 | .001 | 1.5 | .0001 | .5 | " | .5 | 3.1 | 43 |

* All doses were given within 14 min. after administering endotoxin.

Doses given in μg. E, ether; D, decaptitation; N, nembutal

TABLE III. Epinephrine Activity in Isolated Rabbit Ear Associated with Administration of 50 μg of *E. coli* Endotoxin.

| Exp. | Tests before endotoxin | | Tests after endotoxin* | | | | | | Flow, cc/min. | Perfusion pressure, cm $H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dose | Response, mm $H_2O$ | Dose | Response, mm $H_2O$ | Dose | Response, mm $H_2O$ | Dose | Response, mm $H_2O$ | | |
| 1 E | .01 | 15.0 | .01 | 7.0 | .01 | 17.0 | | | 2.3 | 50 |
| 2 E | .001 | 2.5 | .001 | .0 | .001 | .5 | .001 | 1.0 | 3.0 | 47 |
| 3 E | .01 | 124.5 | .01 | 86.5 | .01 | 94.0 | | | 3.0 | 45 |
| 4 E | .001 | 21.0 | .001 | 19.0 | .001 | 9.5 | " | 10.0 | 2.0 | 45 |
| 1 D | " | 16.5 | " | 16.5 | " | 19.0 | | | 3.0 | 42 |
| 2 D | .01 | 109.0 | .01 | 151.0 | | | | | 3.0 | 50 |
| 3 D | .0001 | 1.5 | .0001 | .5 | .0001 | .5 | .0001 | .0 | 3.1 | 37 |
| 1 N | .01 | 8.0 | .01 | 8.0 | .01 | 5.5 | .01 | 12.0 | 3.1 | 50 |
| 2 N | " | 7.5 | " | 5.0 | " | 6.0 | | | 2.0 | 92 |
| 3 N | .001 | 2.0 | .001 | 2.0 | .001 | 3.0 | .001 | 3.5 | 2.8 | 40 |

* All doses were given within 13 min. after administering the endotoxin.
Doses are given in μg. E, ether; D, decaptitation; N, nembutal

of endotoxin was observed only to a slight extent in the presence of barbiturate, and not at all when ether or decapitation without anesthesia was employed to prepare the ear for perfusion, leads us to question whether the effect described by Zweifach, Nagler and Thomas is actually a direct potentiating action of epinephrine. We have no explanation as to how endotoxin might antagonize a barbiturate effect. 2) One point is noteworthy, our preparations in general had a much lower control epinephrine threshold than those reported for the perfused rabbit ear by above mentioned workers. 3) Our results are entirely uniform in showing that no reversal of epinephrine action after the large doses of endotoxin occurred, even with barbiturate anesthesia. We have no explanation to offer for this failure to confirm Zweifach *et al.*

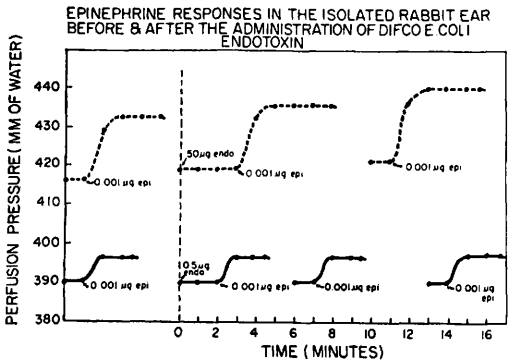


FIG. 1.

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## Post Seizure Mortality Following Electroshock Convulsions in Certain Strains of Mice. (24603)

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Many phenomena associated with electrically-induced convulsive seizures in mice have been studied by investigators. Such factors as seizure threshold and experimental modifications thereof, seizure pattern, characteristics of postictal recovery period and effect of anticonvulsant substances on these various phases have been well described (1-5, and oth-

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