Selective Stimulation of Epinephrine-Responsive Adenyl Cyclase in Mice by Endotoxin (35986)

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Parenterally injected endotoxin causes a number of effects which seem to be associated with selective increases in tissue susceptibility to catecholamines (1). Examples include hyperglycemia and glycogenolysis (2), peripheral vasoconstriction (3), and the production of necrotizing lesions by epinephrine in endotoxin-treated animals (4). Cortisone protects against both the lethal action of endotoxin (5) and the enhanced reactivity to epinephrine (4). The central role of adenyl cyclase in catecholamine function has been well documented (6). Since cortisone profoundly influences the levels of epinephrine-responsive adenyl cyclase in liver (7), as well as the character of endotoxin effects, the possibility of a relationship between endotoxin and epinephrine-responsive adenyl cyclase activity was examined. This report describes a selective activation of mouse epinephrine-responsive adenyl cyclase by *E. coli* endotoxin in liver and spleen.

Materials and Methods. Purified *E. coli.* lipopolysaccharide B (lot 561828) was purchased from Difco. 8-^{14}C-adenosine triphosphate was purchased from Schwarz-Mann. Other biochemicals and reagents were obtained from Sigma. Female Swiss white mice weighing between 15 and 20 g were used. Adrenalectomies were done through a dorsal midline incision; and operated mice were maintained on normal saline and sacrificed after the sixth postoperative day. The livers were perfused *in situ* with a potassium chloride (0.13 M), potassium acetate (0.02 *M*) solution (pH 7.4), and minced and homogenized at ice temperature in a motor driven Potter Elvehjem homogenizer (Teflon on glass) in ⅓ vol of the above solution. The washed particle enzyme was prepared by diluting the whole homogenate with 9 vol of the above potassium chloride/potassium acetate solution and sedimenting the membranes at 10,000*g* for 3 min. The membrane pellet was resuspended in the above solution in a volume equivalent to the original homogenate. Spleens were dissected free of connective tissue, minced, and homogenized in ⅓ vol of the above solution. Adenyl cyclase was assayed using 8-^{14}C-ATP as previously described (8). Endotoxin was administered either intraperitoneally (1 mg/mouse) or by tail vein (100 μg/mouse). Cyclohexamide was administered intraperitoneally at a dose of 3 mg/kg, 30 min prior to endotoxin injection (9).

Results. An increase in epinephrine-responsive adenyl cyclase activity was observed in the livers of mice 3–4 hr after intraperitoneal or intravenous injection of endotoxin (Table I). There was no discernible effect on the glucagon-activated adenyl cyclase in liver. The epinephrine-responsive adenyl cyclase activity was higher (7) and the endotoxin-mediated increase in epinephrine-responsive cyclase was more pronounced in the adrenalectomized animals *in vivo*.

The role of protein synthesis in endotoxin effects was examined by administering cyclohexamide intraperitoneally in a dose sufficient to completely block translation during the entire course of the experimental procedure (9). The cyclohexamide-treated mice exhibited an endotoxin stimulation of the epinephrine-responsive cyclase which was fully comparable to that found in control animals (not illustrated).

Liver cyclase was sensitive to the effects of endotoxin added *in vitro,* which provided

TABLE I. Effects of ip and iv Endotoxin on Liver Adenyl Cyclase Activity in Normal and Adrenalectomized Mice.[a]

Experimental conditions	Adenyl cyclase activity		
	No hormone	Epinephrine (1×10^{-5} *M*)	Glucagon (1.4×10^{-6} *M*)
Intact control	4.7	8.4	38.6
Intact + endotoxin (ip)	4.8	12.6	37.5
Adrenalectomized control	5.0	16.3	36.7
Adrenalectomized + endotoxin			
(ip)	5.3	29.8	39.2
(iv)	5.2	30.7	38.4

[a] The washed particle enzyme was used. Hormones were added (1:10) to the enzyme membrane suspension prior to the addition of the other reaction components (8-^{14}C-ATP (1.32×10^{-3} *M*), aminophylline (6.6×10^{-3} *M*), $MgSO_4$ (3.5×10^{-3} *M*), glycylglycine (4×10^{-2} *M*)). Enzyme activity was assayed for 3 min at 37° and reactions were terminated by boiling for 1 min. Experiments were done in triplicate and values agreed within 5%. Data given are the averages of six determinations. Adenyl cyclase activity is expressed as nanomoles of cAMP/10 mg/10 min.

selective activation of epinephrine-responsive cyclase over the range of concentrations shown (Table II). The spleen was also examined for endotoxin effects *in vitro*. Using adrenalectomized mice, the response of the spleen cyclase to epinephrine was increased threefold by the addition of 0.10 μg/ml of endotoxin (Table II).

The possibility was considered that endotoxin could change the saturation characteristics of the dose–response curve for epinephrine. This might appear either as a change in the saturating concentration required for maximum activation, or a change in the maximum velocity at the normally saturating concentration. The latter explanation appears justified from the data shown in Fig. 1.

Discussion. The above data suggest that endotoxin can selectively increase the epinephrine-responsive adenyl cyclase activity in mouse liver 3–4 hr following parenteral administration. The effect was more pronounced in adrenalectomized mice and was specific for the epinephrine-responsive component of liver adenyl cyclase. The glucagon-responsive component of liver cyclase was entirely unaffected. The addition of endotoxin *in vitro* to suspensions of washed membrane particles from liver or the whole homogenate from spleen prepared from adrenalectomized animals also resulted in a rapid and marked activation of the epinephrine-responsive adenyl cyclase. This effect of endotoxin did not depend on new protein synthesis since the effects were seen *in vivo* and *in vitro* and were not blocked by cyclohexamide. Since the effects of endotoxin *in vivo* survived the procedure of washed particle preparation, this suggests that endotoxin produced a

TABLE II. *In Vitro* Activation of Epinephrine-Responsive Adenyl Cyclase by Endotoxin in Liver and Spleen.[a]

Tissue and endotoxin conc (μg)	Adenyl cyclase activity	
	No hormone	L-Epinephrine bitartrate (1×10^{-5} *M*)
Liver		
Control	5.2	12.8
Endotoxin, 0.1	5.0	25.6
1.0	4.8	20.5
5.0	4.7	18.8
Spleen		
Control	10.8	16.2
Endotoxin, 0.1	10.0	26.2
1.0	9.4	24.5

[a] Endotoxin solutions were prepared in 0.15 *M* NaCl and were mixed with the enzyme suspensions in a ratio of 1 part in 10 to produce the final concentrations shown. Enzyme–endotoxin mixtures were stored on ice for less than 30 min and assayed at 37° for 3 min. Adenyl cyclase activity is expressed as nanomoles of cAMP/10 mg/10 min.

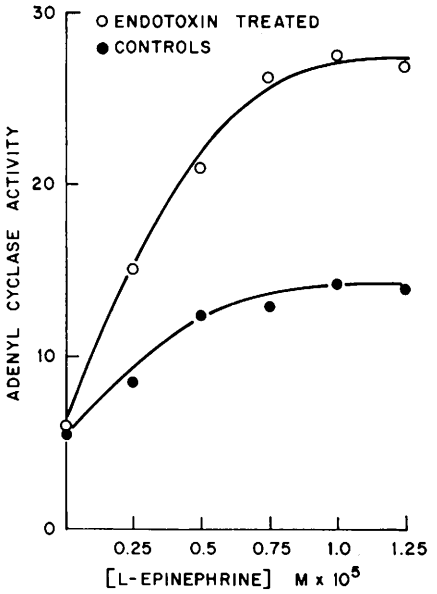

FIG. 1. *In vitro* epinephrine concentration curve using enzymes from normal and endotoxin-treated animals: Adenyl cyclase activity is expressed as nanomoles of cyclic AMP/10 mg of protein/10 min. Endotoxin was injected ip and animals were sacrificed at 4 hr. The livers of 3 animals from endotoxin and control groups were used for preparation of washed particle enzymes. Epinephrine was added to enzyme suspensions to produce the final concentrations shown. Other reaction components and assay concentrations are as in Table I.

stable structural change in cyclase or was firmly bound to the membrane. Our data do not distinguish between these possibilities.

The data indicate that adenyl cyclase may be the initial locus of action of endotoxin and may explain such effects as liver glycogenolysis and the previously described increase in sensitivity to catecholamines (1). Although epinephrine would appear to be necessary for the expression of endotoxin reactions, medullary catecholamines are not necessary for the development of these reactions, since endotoxin alone produced marked changes in the potential epinephrine cyclase activity of adrenalectomized animals as well as *in vitro*. Thus the full expression, but not the initiation, of endotoxin effects require the presence of epinephrine.

Endotoxin has recently been reported to increase the release of neurotransmitter substances at the myoneural junction in the crayfish (10). This effect is consistent with the observations in this report since adenyl cyclase has been found to regulate the release of neurotransmitter at the myoneural junction (11).

Since endotoxin reliably provokes glycogenolysis it would seem reasonable to conclude that the hepatocytes participate, at least to some extent, in the observed enzyme changes. The liver is histologically complex and the observed effects could involve Kupffer's cells, hepatocytes, or other cell populations. It is not known what cells participate in the reaction observed in spleen preparations.

Summary. A marked and selective stimulation of epinephrine-responsive cyclase activity *in vivo* and *in vitro* is caused by *E. coli.* lipopolysaccharide B endotoxin in normal and adrenalectomized mice. The effects are independent of protein synthesis and do not represent a change in the saturation characteristics of the dose–response curve for epinephrine. The effects appear compatible with the known increase in susceptibility to catecholamines in endotoxin-treated animals.

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