Endotoxemia and adrenaline-hyperreactive death in mice

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Summary. Mice given i.v. a sublethal dose of endotoxin in advance died with shock-like symptoms on administration of sublethal adrenaline dose. The lethal adrenaline-hyperreaction induced by endotoxin appeared gradually within a few h, showed maximum response after several h and almost disappeared 24 h after endotoxin administration.

It is well known that bacterial endotoxins have many kinds of biological activities. Recently our interests have been focussed on the relationship between endotoxins and the development of shock, especially on the histamine-sensitizing activity of endotoxin in mice[2,3]. During the course of the study, we encountered the phenomenon that the mice previously given endotoxin through i.v. route died hyperreactive death on a sublethal adrenaline dose. In this paper, we will present an outline of the adrenaline-hyperreactive death in mice with experimentally introduced endotoxemia. Some findings have already been published[4].

Materials and methods. The lipopolysaccharide (LPS) preparation mainly used as endotoxin was *Escherichia coli* LPS (Control No. 573564, Difco Co.) prepared by Westphal's method (W) from *E. coli* 0 111 B4 cells. In addition, both preparation types, Westphal (W) and Boivin (B) type, of LPS derived from some gram-negative bacteria were also obtained from the same company and the *E. coli* LPS (Y) was prepared from *E. coli* 0 111 B4 cell by ether treatment, phenol extraction and ultracentrifugation in this laboratory[5]. Female mice of ddY/S strain, 11-12week-old, were used as in the previous papers[2,3].

Mice were inoculated via tail vein with 0.2 ml of the LPS solutions containing various amounts of LPS in pyrogen-free saline, and i.p. challenged with adrenaline solution in a volume of 0.5 ml at the various periods after or before LPS administration. Deaths were tabulated 18 to 20 h after adrenaline challenge. A control mouse group treated with adrenaline alone ran always with each test as an adrenaline control, and another control mouse group treated with endotoxin alone ran with most tests as an endotoxin control. All glasswares, syringes, and needles were heated for 30 min at 250 °C to exclude pyrogens. As diluent of LPS and adrenaline solution was used pyrogen-free saline confirmed by the pyrogen test method in rabbit. For statistical analysis of lethality, Fisher's exact test[6] was applied.

Results and discussion. The previous paper[3] revealed that the pretreatment with adrenaline inhibited the induction of histamine-hypersensitivity by endotoxin in mice, but it was impossible to know the effect of adrenaline-posttreatment on the induction, because the mice i.v. given a sublethal dose of *E. coli* endotoxin resulted in unexpected acute deaths with shock-like symptoms on the i.p. administration of sublethal adrenaline dose alone before histamine challenge. The new unexpected phenomenon caused us to investigate its reproducibility and the relation between endotoxemia and adrenaline-hyperreactive death.

Experiment 1 of the table shows that the unexpected phenomenon mentioned above was reconfirmed, that is, the lethal rates (11/20 and 14/20) due to adrenaline challenge (100 µg dose/mouse) 2 and 4 h after the i.v. administration of *E. coli* LPS (Y) were significantly larger not only than the rate (0/20) of LPS control, but also than that (4/20) of adrenaline control. The adrenaline control indicated that 100 µg of adrenaline is lethal a certain proportion of mice. In the following experiments, therefore, mice were challenged with 50 µg of adrenaline. The adrenaline-lethality (14/40) of mice was significantly increased 4 h after the LPS administration as compared with those of the control groups (see experiment 2). These facts indicate that mice become hyperreactive to the sublethal dose of adrenaline 2-4 h after the administration of sublethal endotoxin dose. Most of these mice died within 1 h after the adrenaline challenge with shock-like symptoms, and a few mice were found dead 18-20 h after the challenge.

Experiment 3 shows that the mice given i.v. LPS (W) instead of LPS (Y) of *E. coli* also showed the adrenaline-hyperreactive death 4 h after LPS administration, while the mice given i.v. LPS (W) 1 or 2 h after adrenaline administration did not show any adrenaline-hyperreaction. The hyperreaction was inducible only in the endotoxemic state of mice which were i.v. given endotoxin in advance. We provisionally named the above-described phenomenon the lethal adrenaline-hyperreaction induced by endotoxin.

Experiments 4-6 showed the time response in h of the lethal adrenaline-hyperreaction in a range of 1-24 h after

Incidence of lethal adrenaline-hyperreaction due to endotoxin administration[a]

Experiment No.	Endotoxin (LPS) control Preparation[b]	Dose µg/mouse	Death rate	Adrenaline lethality after and before LPS in following periods (h) −2	−1	1	2	3	4	5	6	8	12	18	24	Adrenaline control Death rate
1	*E. coli* (Y)	0.313	0/20[d]				11/20**[e]		14/20****							4/20[f]
2	*E. coli* (Y)	0.313	0/40				3/40		14/40**							0/30
3	*E. coli* (W)	10	0/20	0/20	0/20				5/20**							0/20
4	*E. coli* (W)	100	0/14			0/14	1/14	3/14	4/14*	3/14	4/14*					0/14
5	*E. coli* (W)	100	-			2/14			7/14***		3/14	3/14	3/14	1/14	0/14	0/14
6	*E. coli* (W)	200	0/20						7/20***		6/20**		1/20	1/20	0/20	0/20
7	*S. typhosa* (W)	100	0/20				6/20**		15/20****						3/20	0/20
	S. typhosa (B)	100	0/20				7/20***		11/20****						2/20	
	Sh. flexneri (B)	100	0/20				3/20		8/20***						0/20	

[a]Female mice were i.v. injected with LPS solution. [b]LPS preparations used extracted by Westphal's method (W), Boivin's (B) or Yoshikawa's (Y). [c]Challenge dose of adrenaline: 50 µg/mouse in all experiments except in experiment 1 (100 µg/mouse). [d]Numerator represents the number of dead mice; denominator the total number of mice given LPS alone. [e]Significance of difference in lethality between the LPS-adrenaline groups and the adrenaline control groups was tested according to the Fisher's exact method. Significance level, *5%, **2.5%, ***1% and ****0.1%. [f]Numerator represents the number of dead mice; denominator the total number of mice given adrenaline alone. [g]Numerator represents the number of dead mice; denominator the total number of mice given LPS and adrenaline.

E. coli LPS administration. In experiment 4, both of lethal rates (4/14, 4/14) 4 and 6 h after LPS administration were significantly larger than that (0/14) of adrenaline control. The time response indicated the gradual appearance of the hyperreaction and the peak of response between 4 and 6 h after LPS administration. In experiments 5 and 6, the evident adrenaline-hyperreaction maintained till 6 h after LPS administration, would gradually decrease and finally disappear 24 h thereafter.

In experiments 1-6, the LPS (W) and LPS (B) preparations of *E. coli* were studied. Furthermore, both preparation types, Westphal (W) and Boivin (B) type, of LPS derived from *Salmonella typhimurium, Salmonella typhosa, Shigella flexneri* and *Serratia marcescens* were studied including Bovin type of *E. coli* LPS. Some data are presented as experiment 7 in the table. All preparations in doses of 10-100 µg per mouse always showed the lethal adrenaline-hyperreacting activity 4 h after LPS administration, regardless of the LPS extraction methods and the origin of bacterial cells. Little or no activity was detected 24 h after LPS administration. These data were consistent with those of experiments 4-6.

We arbitrarily adopted the 4-h interval between the endotoxin administration and the challenge of adrenaline for the analysis of dose-response relationship. Thus, we obtained linear dose-response relationships with varying endotoxin doses and a constant adrenaline dose, as well as with varying adrenaline doses and a constant endotoxin dose[4]. The characteristics of these dose-response lines will be presented in detail elsewhere.

The mechanism of the adrenaline-hyperreaction described above is not understood. Some workers reported the release of adrenaline in vivo by endotoxin administration[7-16]. Such a release of adrenaline may be involved in the lethal adrenaline-hyperreaction in endotoxemia. The exogeneous addition of a sublethal adrenaline dose to the possibly increased adrenaline contents in blood by endotoxin may result in the shock-like death of mice. The hypothesis also suggests that the clinical therapeutical use of adrenaline may bring unexpected prognosis to patients in shock accompanying endotoxemia, who may have high adrenaline content in blood.

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Metabolism of hydrazines and hydrazides by the intestinal microflora

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Summary. Intestinal microorganisms are able to effect the metabolic reductive fission of hydrazines but not hydrazides during incubation in vitro.

Since the intestinal microflora is known to be of considerable importance in the metabolism of many azo-compounds, including both food colours[2] and the drugs, neoprontosil[3], salicylazosulphapyridine[4] and phenazopyridine[5] it appeared of interest to establish whether drugs possessing the related hydrazo-linkage were also susceptible to reductive fission by the intestinal microflora. Studies are now reported upon the ability of the microflora of the rat intestine to degrade a number of hydrazo-substituted compounds under conditions of anaerobic incubation in vitro.

The incubations were carried out under anaerobic conditions employing a glucose-peptone-yeast extract medium[6]. Each drug was added to the sterile medium to give a concentration of 0.36 mg/ml; the drug preparations previously being sterilized either by autoclaving under controlled conditions or in the case of labile compounds, by passage through millipore filters. Mixed inoculae of rat intestinal organisms were obtained by sterile section of the rat intestine when a small amount of the withdrawn material was suspended in sterile 0.9% aq. NaCl. Following inoculation the incubation mixtures were held at 37 °C for 7 days under N_2. Appropriate noninoculated controls were incubated under similar conditions. Following incubation the tubes were centrifuged and the supernatants passed through millipore filters and submitted either directly or following a dansylation treatment to TLC. Dansylation was carried out employing a modification of the procedure described by Smith[7]. To 1.0 ml of the supernatant, 0.1 ml of a 1 M solution of sodium bicarbonate was added. A solution of saturated dansyl chloride in acetone was added and the tube sealed and allowed to stand in the dark for 12 h. Acetone (8 ml) was added and the precipitate of protein and inorganic salts removed by centrifugation. The supernatants were then applied to TLC plates for investigation.

The compounds selected for investigation were of the hydrazine (table 1) or hydrazide type (table 2). Following incubation, all of the hydrazine compounds were found to have given rise by reductive fission to primary amines detected on TLC plates as their dansyl derivatives (table 3). Since the intensity of fluorescence and size of each of the spots were similar to those given by the dansyl derivatives of the corresponding primary amines at the same molar levels as the hydrazine substrates initially present it was