ALTERATION OF HISTAMINE, SEROTONIN AND PRIMARY PROSTAGLANDIN IN CASE OF DIARRHEA INDUCED BY ENDOTOXIN AND GASTROINTESTINAL ABSORPTION OF DRUG

EIICHI SAKURAI, NOBORU HIKICHI, AND HIROSHI NIWA*

Tohoku College of Pharmacy, 4-4-1, Komatsushima, Sendai, 983, Japan

(Received July 30, 1984)

Effects of endotoxin on the motility, the permeability of mesenteric blood vessel and endogenous substances (electrolyte (Na, K), histamine, serotonin and primary prostaglandin) in the small intestine of mice were investigated. Diarrhea was induced by intraperitoneal injection of *Salmonella typhosa* endotoxin in mice. It is suggested that the diarrheal action of endotoxin is due to the acceleration of intestinal motility and/or the facilitation of mesenteric blood vessel permeability. On the other hand, from the observation of blood concentration of acetaminophen and sulfisoxazole, inhibition of drug absorption was suggested in cases of diarrhea induced by endotoxin. This inhibition of gastrointestinal absorption is due to the delay of the gastric emptying rate, the increase of intestinal transit time of drug in the absorption site and the acceleration of mesenteric blood vessel permeability. Then it is conceivable that these actions may be mediated by biogenic amines (serotonin and histamine) and primary prostaglandins.

Keywords — endotoxin; diarrhea; intestinal motility; mesenteric blood vessel; gastric emptying rate; gastrointestinal absorption; serotonin; histamine; primary prostaglandin

INTRODUCTION

Endotoxin has many physiological actions on the body. In particular, it is well-known that besides shock and pyrexia, endotoxin produces gram-negative bacterial infection as an opportunistic pathogen, and disseminated intravascular coagulation of an acute type.^{1,2)} However, the effect of endotoxin on the intestine is not known in detail.

On the other hand, it is generally believed that certain types of diarrhea are related to a stimulation of intestinal motility and mesenteric blood vessel permeability, and to an inhibition of water reabsorption. These actions in the small intestine have been reported for various cathartics.³⁻⁵⁾ We previously reported that these actions were mediated by endogenous substances, serotonin, histamine, polyamine,⁶⁾ cholecystokinin⁷⁾ and bile acid.⁸⁾

We have investigated the influence of endotoxin on intestinal action to evaluate the validity of endotoxin as a diarrheal agent, and also the disposition of endogenous substances (electrolyte (Na, K), histamine, serotonin and primary prostaglandin) to confirm a possible linkage between endogenous substances of diarrheal control and occurrence of diarrhea after injection of endotoxin. Furthermore, as a series of our study on the relationship between the diarrhea and gastrointestinal absorption of drug, we also investigated whether the gastrointestinal absorption of drug is altered during the diarrhea induced by endotoxin.

MATERIALS AND METHODS

Animals — Male ddY mice weighing 20 to 25 g were used in this investigation. Standard food (F-2 Funabashi laboratory) was withheld

* To whom correspondence should be addressed.

for 20 h prior to all the experiment except for cathartic effect; there was free access to drinking water.

Cathartic Effect of Endotoxin — Non-diarrheal mice were selected before the experiment. *Salmonella typhosa* endotoxin (Sigma Chemical Co.) was given *i.p.* at doses of 0.25 to 4.0 mg/kg. After the administration of endotoxin, the mice were observed every hour from 0 to 4 h according to the method described by Tsurumi *et al.*⁹⁾

A dose with a cathartic efficiency of 60%, 2.0 mg/kg, was selected to produce the model of diarrhea. All mice developing diarrhea at 2 h after the injection were used as diarrheal mice for the following experiment.

Rectal Temperature — Non-diarrheal and -feverish mice were selected before the experiment. After the intraperitoneal injection of endotoxin (2.0 mg/kg), rectal temperature was measured every hour from 0 to 5 h by thermistor telethermometer (Natsume Ltd., type 1-A-3046).

In Vivo Experimental Method — Drugs used in this experiment were acetaminophen which is used as the test drug of gastric emptying function, and sulfisoxazole which is insoluble in water. Immediately after intraperitoneal injection of endotoxin (2.0 mg/kg), acetaminophen dissolved in water (8 mg/ml), or sulfisoxazole suspended in water (8 mg/ml) was orally administered by stomach tube at a dose of 200 mg/kg to the mice. Moreover, acetaminophen (200 mg/kg), or sulfisoxazole (200 mg/kg) was orally administered in diarrheal mice. At different times after injection of drug, mice were decapitated, and the blood was collected. The concentration of acetaminophen in blood was determined by the Dye method.¹⁰⁾ The blood sulfisoxazole concentration was also determined according to the method described by Kakemi *et al.*¹¹⁾

Propulsive Motility of the Digestive Tube — 1) Gastric Emptying Time: Phenol red (2.5 mg/kg) was orally administered simultaneously with endotoxin, and 2 h after endotoxin. At 15 min after the oral administration of phenol red, the

amount of phenol red remaining in the stomach was determined.

2) Passage of Charcoal Meal in the Small Intestine: At 2 h after the administration of endotoxin, the mice received 0.2 ml charcoal meal per 20 g mouse body weight. The small intestinal tract was quickly removed, and the passage of charcoal meal in intestinal tract was measured. Intestinal motility was expressed as the percentage of the length traversed by the marker to the total length.

Mesenteric Blood Vessel Permeability — The procedures were employed as reported previously.⁶⁾ Under anesthesia with sodium pentobarbital (40 mg/kg *i.v.*), the small intestine was looped following a midline abdominal laparotomy, and then pontamine sky blue (0.1 ml/10 g mouse body weight) was intravenously administered. The dye leaking into the lumen of the small intestine was collected for a 1-h period after being given the pontamine sky blue. The dye concentration was determined by the method described elsewhere.⁶⁾

Biochemical Analysis — 1) Determination of Na and K in the Serum: Electrolyte (Na and K) level was determined by a flame photometer (Corning Ltd., type 435).

2) Determination of Serotonin and Histamine in the Blood and Small Intestine: The blood and small intestine were homogenized in 0.4 N perchloric acid containing 2 mM ethylenediaminetetra acetic acid (EDTA)-Na and centrifuged. Serotonin was determined as described by Snyder *et al.*¹²⁾ and histamine was measured according to the method described by Endo and Ogura.¹³⁾

3) Determination of Prostaglandin E and F Group in the Small Intestine: Prostaglandin E and F in the small intestine were separated by silica gel column chromatography and determined by bioassay according to Takeuchi *et al.*¹⁴⁾

RESULTS

Cathartic Effect

Control mice developed diarrhea with endotoxin treatment. Endotoxin at doses of 0.5 to 2.5

TABLE I. *Cathartic Effect in Mice of Endotoxin*

Dose (mg/kg, <i>i.p.</i>)	Time (h)				
	0–1	1–2	2–3	3–4	0–4
0.25	0/10	0/10	0/10	0/10	0/10
0.50	2/10	0/10	2/10	0/10	2/10
1.00	0/10	4/10	0/10	0/10	4/10
1.50	2/10	4/10	0/10	0/10	4/10
1.80	0/10	4/10	2/10	0/10	6/10
2.00	0/10	6/10	0/10	2/10	6/10
2.35	2/10	2/10	2/10	0/10	6/10
2.50	2/10	6/10	4/10	2/10	8/10
3.00	2/10	0/10	0/10	2/10	4/10
3.50	0/10	4/10	4/10	2/10	4/10
4.00	2/10	2/10	0/10	0/10	2/10

Endotoxin: Salmonella typhosa endotoxin.

mg/kg (*i.p.*) caused diarrhea in a dose-dependent manner. In contrast, large doses of endotoxin (4.0 mg/kg, *i.p.*) inhibited the occurrence of diarrhea. The occurrence of diarrhea concentrated within 2 to 3 h after the administration of endotoxin (Table I). Fever was not observed between 1 and 5 h after intraperitoneal injection of endotoxin (2.0 mg/kg) (Fig. 1).

The Blood Concentration of Drug

The blood concentrations of acetaminophen and sulfisoxazole were shown in Figs. 2 and 3. The average time courses of acetaminophen and sulfisoxazole in the blood of mice treated simultaneously with endotoxin were not significantly different from that in the control. But, in the cases associated with diarrhea, the mean blood concentrations of acetaminophen and sulfisoxazole were significantly lower than that in the control.

Propulsive Motility of the Digestive Tube

At 15 min (in initial stage of diarrhea) and 2 h (during diarrhea) after the administration of endotoxin, the percentage of phenol red recovery in the stomach was significantly higher than the control group (Fig. 4). The passage of charcoal meal in both stages of diarrhea was significantly accelerated in comparison with the control (Fig. 4).

FIG. 1. *Effect of Endotoxin on Rectal Temperature in Mice*

Mice were intraperitoneally injected with 2.0 mg/kg of *Salmonella typhosa* endotoxin. Each point represents the mean response of 6 mice.

Mesenteric Blood Vessel Permeability

As shown in Table II, the output of pontamine sky blue into the small intestinal tract of diarrheal mice induced by endotoxin was $17.5 \pm 0.6 \mu\text{g}/10 \text{ g}$ mouse body weight (mean $\pm$ S.E.), as compared with $9.6 \pm 1.6 \mu\text{g}/10 \text{ g}$ mouse weight in the control ($p < 0.05$). This result demonstrated that application of endotoxin significantly increased mesenteric blood vessel permeability.

Electrolytes, Biogenic Amines and Prostaglandins Level in the Tissues after Endotoxin Injection

There was no significant difference in the electrolyte (Na and K) level of the serum between endotoxin-treated and non-treated mice

FIG. 2. Effect of Endotoxin on the Blood Concentration of Acetaminophen

Acetaminophen (200 mg/kg) was orally administered simultaneously with endotoxin, and in endotoxin-induced diarrheal mice. Each result represents the mean $\pm$ S.E. of 6 mice.

—●—: control mice, —■—: simultaneous treated mice with endotoxin, —△—: diarrheal mice.

FIG. 3. Effect of Endotoxin on the Blood Concentration of Sulfisoxazole

Sulfisoxazole (200 mg/kg) was orally administered simultaneously with endotoxin, and in endotoxin-induced diarrheal mice. Each result represents the mean $\pm$ S.E. of 6 mice.

—●—: control mice, —■—: simultaneous treated mice with endotoxin, —△—: diarrheal mice.

FIG. 4. Percentage of Phenol Red Recovered from the Stomach and Passage of Charcoal Meal in the Small Intestine of Mice after Ingestion of Endotoxin

Salmonella typhosa endotoxin was given i.p. in 2.0 mg/kg. The content of phenol red in the stomach was determined 15 min after phenol red was orally administered in initial stage diarrhea and during diarrhea induced in mice by endotoxin. The passage of charcoal meal was determined 15 min after charcoal meal was orally administered in initial stage diarrhea and during diarrhea induced in mice by endotoxin. Each result represents the mean $\pm$ S.E. of 6 mice. a) Differences are significant at $p < 0.01$. b) Differences are significant at $p < 0.05$. c) 15 min after administration of endotoxin d) 2 h after administration of endotoxin

(Table III). Therefore, it is suggested that enteropooling (the accumulation of fluid in the small intestine) was not induced by endotoxin. On the other hand, the amount of serotonin and histamine in the blood and small intestine of mice are shown in Table IV. The serotonin level in the blood significantly decreased after endotoxin treatment, but the level of serotonin in the small intestine increased in comparison with the control. The histamine level increased significantly in the small intestine of diarrheal mice. The application of endotoxin resulted in the rapid release of histamine from the small intestine. Table V shows prostaglandin E and F content in the small intestine of diarrheal mice. The prostaglandin E and F levels of the small intestine decreased from 35.55 ± 2.71 and 18.57 ± 1.61 ng/g (mean $\pm$ S.E.) initially to 22.27 ± 1.40 and 9.08 ± 1.96 ng/g at 2 h after injection of endotoxin in control mice, respectively. This result suggests that the biosynthesis of prostaglandin E and F in the small intestine is inhibited in diarrhea.

DISCUSSION

Salmonella typhosa endotoxin caused diarrhea

without fever at dose above 0.50 mg/kg *i.p.* in mice. The occurrence of diarrhea concentrated within 2 to 3 h of treatment, but was inhibited at dose above 2.5 mg/kg *i.p.*

It is generally believed that the induction of diarrhea is related to a stimulation of intestinal motility and to acceleration of intestinal permeability. From this effect of endotoxin to the mouse intestine, we suggested that the diarrheal action of endotoxin was due to the stimulation of intestinal motility in initial stage of diarrhea after administration of endotoxin. This acceleration lasted for more than 2 h. Further, endotoxin markedly increased the permeability of mesenteric blood vessel and promoted the occurrence of diarrhea.

On the other hand, we have observed the disposition of serotonin, histamine and primary prostaglandin in the tissue, and studied the physiological significance of these substances in the case of diarrhea induced by endotoxin. It is well-known that histamine or serotonin has the ability to accelerate permeability, secretion and motility in the gastrointestinal tract.^{15,16)} These facts proved that vascular permeability in the small intestinal membrane observed in diarrhea is ac-

TABLE II. *The Output PSB into the Small Intestinal Tract in Diarrheal Mice Induced by Endotoxin*

	PSB output (μ g/10 g mice body weight)
Control	9.6 ± 1.6
In endotoxin-induced diarrheal mice	$17.5 \pm 0.6^a)$

Salmonella typhosa endotoxin was given in *i.p.* in 2.0 mg/kg. Each result represents the mean $\pm$ S.E. of 6 mice. a) Differences are significant at $p < 0.05$. PSB: pontamine sky blue.

TABLE III. *Electrolyte (Na, K) Levels of Serum in Endotoxin Induced-Diarrheal Mice*

	Initial stage of diarrhea		In diarrhea	
	Na (meq/l)	K (meq/l)	Na (meq/l)	K (meq/l)
Control	158.0 ± 0.4	8.0 ± 0.1	158.0 ± 0.4	8.4 ± 0.1
Endotoxin	157.0 ± 0.5	8.0 ± 0.1	156.5 ± 0.3	8.0 ± 0.2

Salmonella typhosa endotoxin was given *i.p.* in 2.0 mg/kg. Each result represents the mean $\pm$ S.E. of 6 mice.

accompanied by the release of endogenous histamine from the small intestine. In this study, however, we could not obtain the apparent relationship between the increase of intestinal motility and the disposition of serotonin and histamine in the small intestine during diarrhea induced by endotoxin. In addition, it is conceivable that the diarrheal action of endotoxin is due not to the enteropooling, because there is no variation in the serum electrolyte (Na, K) level in diarrhea following injection of endotoxin. Moreover, the determination of prostaglandin E and F has been carried out to study the role of prostaglandin in diarrhea. The presence of both antisecretory and cytoprotective activities was initially found in prostaglandins which were distributed in the gastrointestinal tract in many species.¹⁷⁻¹⁹⁾ Primary prostaglandins protected the mucosa of the intestine or stomach. This physiological property is independent of the control of intestinal or gastric permeability. In our experiment, inhibition of prostaglandin biosynthesis was observed in diarrhea induced by endotoxin. This phenomenon purports that the cytoprotective action of prostaglandins for the intestinal

mucosa is collapsed. Accordingly it is conceivable that the collapse of cytoprotection produced the obstruction of the small intestinal mucosa and promoted it. However, contrary to our experiment, Vischer and Casals-Stenzel²⁰⁾ have recently shown that a diarrheal action of castor oil is due to a stimulation of endogenous prostaglandin biosynthesis. On the other hand, the release of various prostaglandins from the tissues following endotoxin administration has been also reported in other species.²¹⁾ Therefore, further studies may be necessary to elucidate the relationship between the quantitative alteration of prostaglandin and the occurrence of diarrhea.

Moreover, the gastric emptying rate, intestinal transit time or intestinal permeability is a well-known factor which have influence on the gastrointestinal absorption of drugs. From the observation of blood concentration of acetaminophen and sulfisoxazole, inhibition of drug absorption is suggested in cases of diarrhea induced by endotoxin. This inhibition of gastrointestinal absorption is due to the delay of the gastric emptying rate and the quickness of intestinal transit time of drug in the absorption site. Fur-

TABLE IV. *Serotonin and Histamine Levels of Blood and Small Intestine in Endotoxin-Induced Diarrheal Mice*

	Serotonin levels		Histamine levels	
	Blood (nmol/g)	Small intestine (nmol/g)	Blood (nmol/g)	Small intestine (nmol/g)
Control	0.47 ± 0.03	15.80 ± 0.71	0.35 ± 0.03	2.88 ± 0.35
Endotoxin	0.30 ± 0.01 ^{a)}	22.24 ± 2.36 ^{a)}	0.50 ± 0.04 ^{a)}	1.88 ± 0.39 ^{a)}

Salmonella typhosa endotoxin was given i.p. in 2.0 mg/kg. Each result represents the mean ± S.E. of 6 mice. ^{a)} Difference are significant at $p < 0.05$.

TABLE V. *Prostaglandin E and F Levels of Small Intestine in Endotoxin-Induced Diarrheal Mice*

	Prostaglandin E group (ng/g)	Prostaglandin F group (ng/g)
Control	35.6 ± 2.7	18.6 ± 1.6
In endotoxin-induced diarrhea	22.3 ± 1.4 ^{a)}	9.1 ± 2.0 ^{a)}

Salmonella typhosa endotoxin was given i.p. in 2.0 mg/kg. Each result represents the mean ± S.E. of 6 mice. ^{a)} Difference are significant at $p < 0.05$.

thermore, it is suggested that the acceleration of mesenteric blood vessel permeability promotes the inhibition of gastrointestinal absorption of drugs.

In conclusion, the present studies demonstrate in mice that the diarrheal action of endotoxin is due to the acceleration of intestinal motility and/or the facilitation blood vessel permeability. Then these action have influence on drug absorption, and may be mediated by biogenic amines or prostaglandins. However, it is conceivable that this diarrheal action may be due to other unknown gastrointestinal dysfunctions. Further work is required to elucidate the possible diarrheal action of endotoxin.

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