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Role of Interleukin-1β, Interleukin-6, and TNF-α in Intestinal Maturation Induced by Dietary Spermine in Rats

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In the present investigation, the authors aimed to evaluate the role of cytokines in intestinal postnatal maturation induced by dietary polyamines. Neonatal rats were administered either saline or spermine (8 μmol) orally. Spermine increased interleukin-1β (IL-1β), IL-6, and TNF-α plasma concentration. The maximum concentrations of IL-1β, IL-6, and TNF-α were, respectively, observed at 4, 4, and 8 h posttreatment. Intraperitoneal (ip) injection of IL-1β increased the specific activity of sucrase in whole small intestine, whereas the specific activities of maltase and lactase were significantly enhanced only in the jejunum. IL-6 elicited sucrase and increased maltase specific activity in the whole small intestine, but lactase specific activity was not affected. TNF-α had no effect on sucrase and maltase specific activity, but a slight augmentation of lactase specific activity was detected in the jejunum. Spermine and spermidine content in the intestine was increased by ip injection of IL-1β and IL-6. Corticosterone secretion was elevated by single ip injection of IL-1β, IL-6, or TNF-α. These findings suggest that spermine could induce postnatal intestinal development and corticosterone secretion through a cytokine-dependent mechanism.

Key Words: Spermine; cytokines; corticosterone; intestine; suckling rats.

Introduction

The polyamines, spermidine, spermine, and their precursor, putrescine, are polycationic compounds found in high concentrations in all eucaryotic cells *(1)*. Their intracellular levels are dependent on the activity of ornithine decarboxylase (ODC), one of the initial rate-limiting enzymes in polyamine synthesis *(2)*. Numerous studies have shown that the polyamines are implicated in many metabolic processes. Indeed, polyamine synthesis usually precedes DNA and protein synthesis *(3)*.

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During the postnatal development of rodent small intestine, the mucosa undergoes substantial modifications affecting its structural and functional characteristics. Histologically, the large supranuclear vacuoles contained in immature enterocytes, disappear at weaning *(4)*. Biochemically, the mucosal ODC activity and polyamine content increase *(5)*. During the same period, some intestinal enzyme specific activities such as that of lactase decrease whereas others such as that of sucrase and of maltase increase *(6–8)*.

Numerous studies report that postnatal digestive tract maturation is controlled by hormonal factors such as corticosterone *(9)*, thyroxin *(10)*, and insulin *(11)*. Polyamines are implicated in this phenomenon as well. Indeed, it is now established that oral administration of spermine to neonatal rats precociously induces structural and biochemical changes in the small intestine, which are characteristic of natural postnatal intestinal maturation *(12–14)*. The mechanism involved in this process is not yet clearly defined although it is known that polyamines are normal milk constituents and that these substances are more concentrated in rat solid food than in rat milk *(15,16)*.

Previous studies in the authors' laboratory *(17)* showed that spermine, administered orally to neonatal rats, induces the secretion of corticosterone and adrenocorticotropic hormone (ACTH). This is not the case for spermine administered intraperitoneally. The same studies showed that adrenalectomy reduced spermine-induced precocious intestinal maturation *(18)*. Otherwise, the parenteral administration of this polyamine has no effect on intestinal maturation *(18,19)*. It is, therefore, suggested that spermine stimulates the secretion of an intestinal factor that activates the pituitary-adrenal system.

There is increasing evidence for a bidirectional network between the neuroendocrine and immune systems *(20,21)*. Interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor (TNF)-α, polypeptides predominantly produced by monocytes and macrophages, have been reported to activate the pituitary-adrenal axis *(22–24)* by stimulating the CRF-containing cells *(25)*. These cytokines were identified within the intestinal mucosa *(26)* and were suggested to be important modulators of intestinal functions. In order to assess the possible role of IL-1β, IL-6, and TNF-α as a link between the ingestion of spermine and the activation of the pituitary–adrenal axis, spermine was

administered orally to neonatal rats and the changes of plasma concentrations of IL-1β, IL-6, and TNF-α were analyzed. These cytokines were also injected ip to the rats and their effects on intestinal functions and corticosterone secretion were examined.

Results

Influence of Spermine Ingestion on Cytokine Plasma Concentration

The effect of spermine on IL-1β, IL-6, and TNF-α blood concentrations 2, 4, 6, and 8 h postadministration is shown in Fig. 1. Compared with the control values, spermine significantly elevated plasma levels of IL-1β, IL-6, and TNF-α. The maximum concentrations of IL-1β, IL-6, and TNF-α were observed at 4, 4, and 8 h posttreatment, respectively, and were approximately two or three times greater than those observed in control rats. At 8 h posttreatment, spermine significantly reduced the IL-1β plasma concentration. These results were obtained by using bioassays. With enzyme linked immunosorbent assay (ELISA) kits, comparable results were obtained. Nevertheless, the second peak of TNF-α concentration observed 8 h after the experiment beginning was noted 10 h after the latter, and the peak of IL-1β concentration was observed 2 h after the beginning of the experiment, whereas the minimal concentration of IL-1β was recorded 6 h after the beginning of the experiment.

Influence of IL-1β, IL-6, and TNF-α on Intestinal Development

As illustrated in Fig. 2, IL-1β, administered ip, significantly increased the specific activity of sucrase in the proximal (jejunum) and distal (ileum) parts of the small intestine. It enhanced the specific activity of maltase and lactase in the jejunum. The length, the weight, and the spermidine content in both jejunum and ileum were significantly increased by IL-1β treatment (Tables 1 and 2). The latter had no effect on animal weight or on the intestinal DNA, protein, putrescine, and spermine content (Tables 1 and 2).

The small intestine of the rats treated with IL-6 (Table 3) showed an increase in the specific activity of sucrase and maltase. The specific activity of lactase was not modified by this treatment. The intestine length, spermidine content in the ileum, and putrescine content in the whole intestine were significantly greater in rats treated with IL-6 than in animals receiving saline alone (Tables 2 and 4). The animal weight as well as the intestinal DNA and protein content were not significantly modified by intraperitoneal injection of IL-6 (Table 4).

Intraperitoneal administration of TNF-α (Table 3) to rats induced a slight increase in the specific activity of lactase, but had no effect on the specific activity of sucrase and maltase.

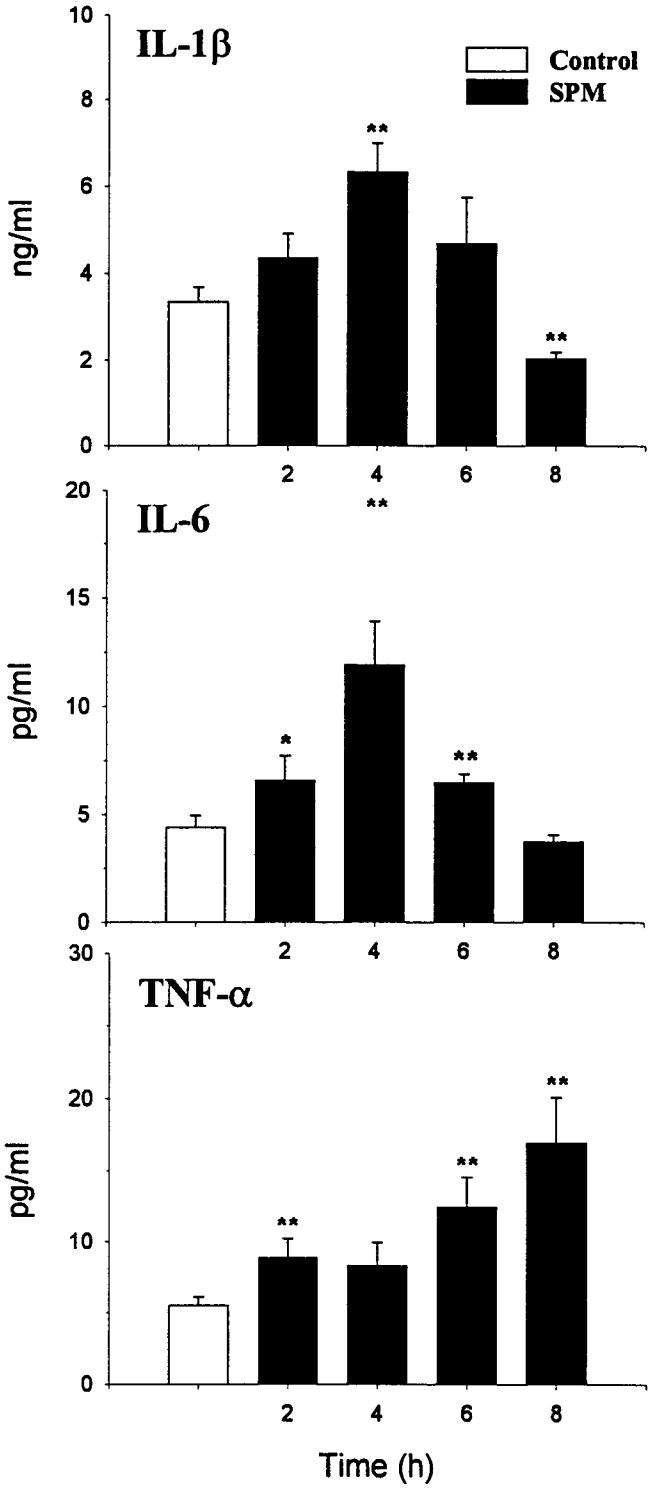

Fig. 1. Effect of spermine (SPM) on the plasma concentration of IL-1β, IL-6, and TNF-α. Eleven day-old rats ingested a single dose of spermine (8 μmol/50 μL of saline). Control animals received 50 μL of saline. The animals were killed 2, 4, 6, and 8 h after the beginning of the experiment. Each bar presents the mean ± SEM of five animals. $^{**}p < 0.01$ vs control.

Influence of IL-1β, IL-6, and TNF-α on Corticosterone Plasma Concentration

The effects of IL-1β, IL-6, or TNF-α on corticosterone blood concentration 2 h after the ip administration of

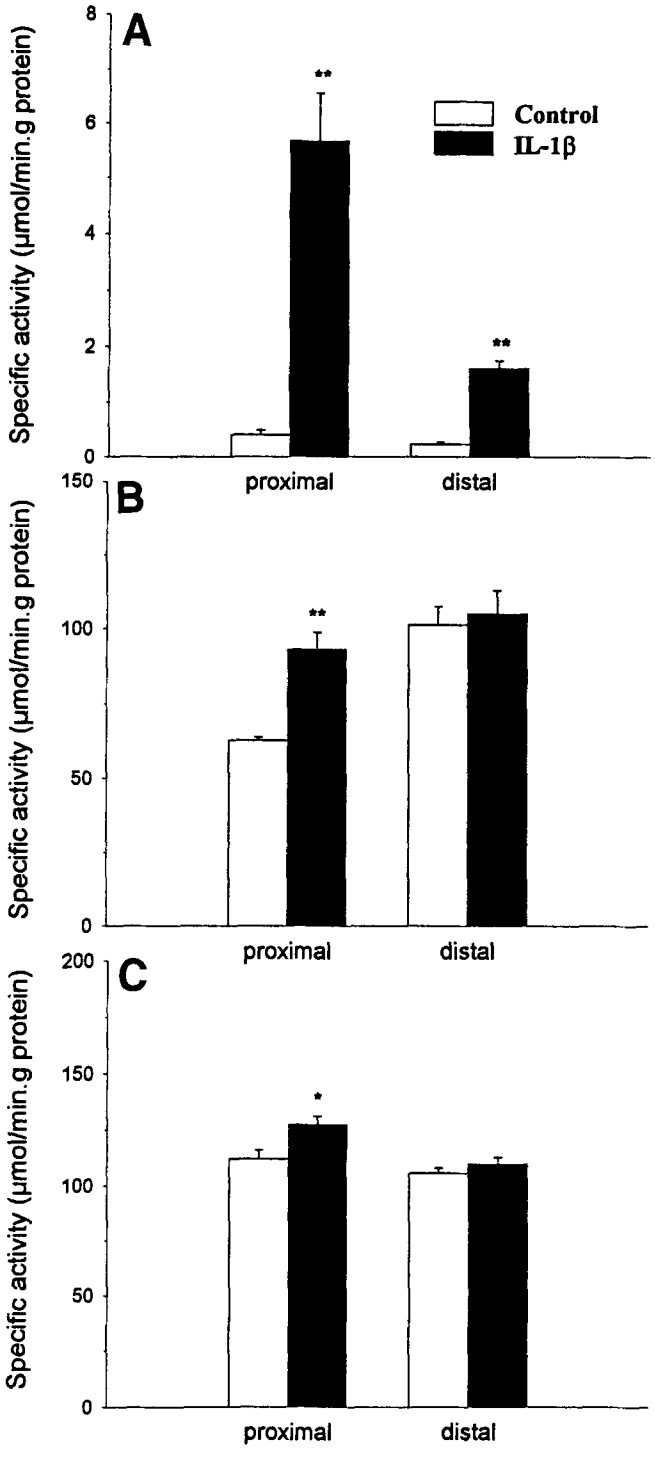

Fig. 2. Effect of IL-1β on the specific activity of sucrase **(A)**, maltase **(B)**, and lactase **(C)** contained in the proximal (jejunum) and distal (ileum) small intestine. Eleven day-old rats were intraperitoneally injected with IL-1β (6 µg/kg body weight), three times per day, for 3 d. Control animals received 100 µL of saline. The animals were killed on their fourteenth postnatal day. Each bar presents the mean ±SEM of five animals. $*p < 0.05$, $**p < 0.01$ vs control.

cytokines are shown in Fig. 3. The data clearly indicate that administration of IL-1β, IL-6, or TNF-α elevated plasma concentration of corticosterone. IL-1β was the most potent of the three cytokines in inducing this phenomenon.

Discussion

Dufour et al. *(12)* and Georges et al. *(13)* showed that spermine administered per os to suckling rats precociously induces structural and biochemical changes in the small bowel that are identical to the natural modifications occurring at weaning in these animals. Although these observations were confirmed by two independent laboratories *(14,27)*, the mechanism involved in this phenomenon is yet unclear. Previous reports *(17,19)* demonstrated that oral administration of spermine increases the plasma ACTH and corticosterone concentration, but that the parenteral injection of this polyamine had no effect. Furthermore, it was shown that the intestinal response to spermine is strongly diminished by adrenalectomy *(18)*. It was, therefore, suggested that spermine ingestion by unweaned rats stimulates the secretion of intestinal factors in blood circulation. These factors may increase the production of corticosterone, which induces intestinal maturation as reported in the literature *(10)*. In the current investigation, it is aimed to determine the nature of this intestinal substance.

Several factors produced by the immune system are known to have a stimulatory effect on the hypophysial-adrenal axis: IL-1β, IL-6, and TNF-α are reported to stimulate this axis in rats and mice *(22–24)*. The current results clearly demonstrate that the ingestion of spermine produced increases in IL-1β, IL-6, and TNF-α plasma concentration in unweaned rats. The peak responses of IL-1β and IL-6, appearing within 4 h after spermine administration, corresponded to the peaks of plasma corticosterone and ACTH concentration observed after the same treatment *(17)*. Other results (not presented), obtained by using ELISA kits, showed that IL-1β peak could be observed a little earlier, but this observation could come from differences existing between experiments (other litters, other animals, other period of the year...). Although the most common effect of IL-6 is to inhibit TNF-α secretion, it has been shown that IL-1β and IL-6 were also able to increase this last phenomenon *(28)*. Consequently, both these last observations indicate that the action of spermine on TNF-α release could be mediated by IL-1β and/or IL-6. Figure 1 shows that 8 h after the spermine treatment [or 6 h in another experiment (*see* above: ELISA Results)], plasma concentration of IL-1β was significantly reduced compared with the control values. This could be explained by the immunoregulatory feedback that exists between the cytokines and glucocorticoid hormones *(29,30)*. Another increase, not only of TNF-α concentration (Fig. 1), but also of IL-1β concentration (in another experiment, result not shown), occurring 8 to 10 h after spermine ingestion was recorded confirming the immunoregulatory feedback suggested above (ELISA Results) and reported previously *(17,19)* for plasma corticosterone and ACTH concentration in similar experimental conditions.

There are several reports indicating that IL-1β, IL-6, and TNF-α administration increases ACTH and corticosterone

Table 1
Effect of IL-1β on Body Weight, Intestine Length, Intestine Weight, Intestinal Protein, and DNA Content

	Body weight (g)	Intestine length (cm/g b.wt)	Intestine weight (mg/g b.wt)		Protein content (mg/g w.wt)		DNA content (mg/w.wt)	
			Proximal	Distal	Proximal	Distal	Proximal	Distal
Control	27.02 ± 0.75	1.38 ± 0.03	12.01 ± 0.24	8.77 ± 0.27	113.8 ± 5.3	103.2 ± 3.4	5.91 ± 0.16	6.41 ± 0.27
IL-1	25.45 ± 0.52	1.58** ± 0.03	13.55* ± 0.56	10.04** ± 0.26	101.9 ± 5.6	104.8 ± 4.1	6.52 ± 0.34	6.56 ± 0.19

Eleven day-old rats were intraperitoneally injected with IL-1β (6 μg/kg b. wt.), three times per day, for 3 d. Control animals received 100 μL of saline. The animals were killed on their fourteenth postnatal day. The results are presented as the mean ± SEM of five animals. $*p < 0.05$, $**p < 0.01$ vs control; b. wt = body weight, w. wt = wet weight.

Table 2
Effect of IL-1β or IL-6 on the Polyamine Content in the Proximal (A) and Distal (B) Small Intestine

A				
Polyamine	Control	IL-1β	Control	IL-6
Putrescine	3.00 ± 0.29	3.37 ± 0.18	2.67 ± 0.24	4.56 ± 0.39*
Spermidine	10.20 ± 0.22	11.43 ± 0.53*	8.33 ± 0.53	7.43 ± 0.28
Spermine	7.79 ± 0.19	8.02 ± 0.43	5.64 ± 0.48	5.43 ± 0.39
B				
Polyamine	Control	IL-1β	Control	IL-6
Putrescine	4.84 ± 0.32	4.14 ± 0.36	3.20 ± 0.43	5.22 ± 0.27*
Spermidine	8.82 ± 0.30	9.98 ± 0.75*	7.06 ± 0.29	9.46 ± 0.29**
Spermine	7.48 ± 0.28	7.74 ± 0.28	5.49 ± 0.43	6.07 ± 0.18

Eleven day-old rats were intraperitoneally injected with IL-1β (6 μg/kg b. wt.) or IL-6 (20 μg/kg b. wt.), three times per day, for 3 d. Control animals received 100 μL of saline. The animals were killed on their fourteenth postnatal day. The results are presented as nmol · g^{-1} of proteins and presented as the mean ± SEM of five animals. $*p < 0.05$, $**p < 0.01$ vs control; b.wt = body weight, w. wt = wet weight.

plasma concentration in adult animals *(22,23)*. Furthermore, O'Grady et al. *(24)* proved that IL-1β increases ACTH and corticosterone secretion in 10-d old rats. Such observations were also made under experimental conditions (Fig. 3), although IL-1β induced a more intense effect than IL-6 and TNF-α. Assuming that this effect is mediated in the same way as in adult animals, i.e., through an increasing CRF release *(31)*, these observations show that the pituitary–adrenal system was already mature enough in suckling rats to respond to the stimulatory effect of immunological factors.

Macrophages and lymphocytes are the major sites of cytokine synthesis. IL-1β-like immunoreactivity has also recently been identified in the gastrointestinal tract *(26,32)*. Although other hypotheses can be formulated from these observations and from the current data, it is suggested that the spermine ingestion by unweaned rats stimulates the secretion of IL-1β, IL-6, and/or TNF-α, from the gut-associated-lymphoid-tissue (GALT) system. Afterwards, these substances appear in the general blood circulation, and increase production and release of corticosterone, which triggers the intestinal maturation process according to the well-known action of glucocorticoids on this phenomenon *(9,10)*. This possibility is highly probable because the current observations show that ip administration of IL-1β or IL-6 precociously changes the disaccharidase specific activities and growth of the small bowel although these effects are weak compared to those resulting from spermine ingestion on these biochemical parameters *(12–14,18)*. This fact could be caused by the experimental conditions used (cytokine doses, treatment duration...), and/or the fact that cytokines are not the sole factor intervening in the intestinal maturation induced by spermine.

The current results demonstrated that IL-1β and IL-6 increase the concentration of corticosterone in plasma. These cytokines also increased the intracellular polyamine concentration (Table 2). As IL-1β activates the ODC contained in intestinal epithelial cells in vitro *(33)*, it is possible

Table 3
Effect of IL-6 or TNF-α on the Specific Activity of Disaccharidases in the Proximal (A) and Distal (B) Small Intestine

A				
Disaccharidase	Control	IL-6	Control	TNF-α
Sucrase (nmol/min · g protein)	ud	0.50 ± 0.03**	ud	ud
Maltase (μmol/min · g protein)	29.59 ± 1.33	38.62 ± 1.64*	44.10 ± 1.99	50.60 ± 2.58
Lactase (μmol/min · g protein)	64.02 ± 3.79	59.34 ± 2.48	130.91 ± 3.56	138.45 ± 3.68
B				
Disaccharidase	Control	IL-6	Control	TNF-α
Sucrase (nmol/min · g protein)	ud	0.12 ± 0.01**	ud	ud
Maltase (μmol/min · g protein)	44.64 ± 1.74	55.73 ± 1.04**	96.11 ± 6.78	116.28 ± 8.93
Lactase (μmol/min · g protein)	75.42 ± 3.64	73.67 ± 2.80	111.50 ± 4.03	123.68 ± 2.24*

Eleven day-old rats were intraperitoneally injected with IL-6 (20 μg/kg b. wt.) or TNF-α (20 μg/kg b. wt.), three times per day, for 3 d. Control animals received 100 μL of saline. The animals were killed on their fourteenth postnatal day. The results are presented as the mean ± SEM of five animals. *$p < 0.05$, **$p < 0.01$ vs control; ud = undetectable; b. wt = body weight.

Table 4
Effect of IL-6 on Body Weight, Intestine Length, Intestine Weight, Intestinal Protein, and DNA Content

	Body weight (g)	Intestine length (cm/g b.wt)	Intestine weight (mg/g b.wt)		Protein content (mg/g w.wt)		DNA content (mg/w.wt)	
			Proximal	Distal	Proximal	Distal	Proximal	Distal
Control	32.25 ± 0.47	1.24 ± 0.01	11.53 ± 0.20	8.93 ± 0.21	140.1 ± 5.42	139.3 ± 1.15	5.13 ± 0.09	5.98 ± 0.09
IL-6	30.16 ± 0.92	1.40** ± 0.03	13.72* ± 0.17	0.44** ± 0.35	139.3 ± 3.3	135.7 ± 2.7	5.03 ± 0.16	5.83 ± 0.59

Eleven day-old rats were intraperitoneally injected with IL-6 (20 μg/kg b. wt.), three times per day, for 3 d. Control animals received 100 μL of saline. The animals were killed on their fourteenth postnatal day. The results are presented as the mean ± SEM of five animals. *$p < 0.05$, **$p < 0.01$ vs control; b. wt = body weight, w. wt = wet weight.

that the increase of polyamine content induced by IL-1β and IL-6 is a consequence of a direct action of these cytokines on the intestinal ODC activity. Nevertheless, an indirect action of IL-1β and IL-6 on the intestinal polyamine concentration through an increase in plasma concentration of corticosterone cannot be excluded. The latter is known to be an activator of ODC *(34)*.

The indirect action of spermine, via factors other than corticosterone, on the enterocytes is not excluded: the receptors for the cytokines are present on the intestinal epithelial cells *(31)*; the administration of IL-1β to the rats induced release of insulin and prostaglandins *(35)*, which are involved in the postnatal intestinal maturation.

From another point of view, it cannot be affirmed that spermine acts directly on cells producing cytokines and exclude that spermine acts directly on the enterocytes. Other experiments have to be programmed in order to solve these problems.

Materials and Methods

Animals

Wistar rats were mated and bred in an animal room with a 12 h light:darkness cycle. They had free access to water and food. The date of birth was designated as d 0 and litter sizes were restricted to 10–12 pups on d 2. All animal

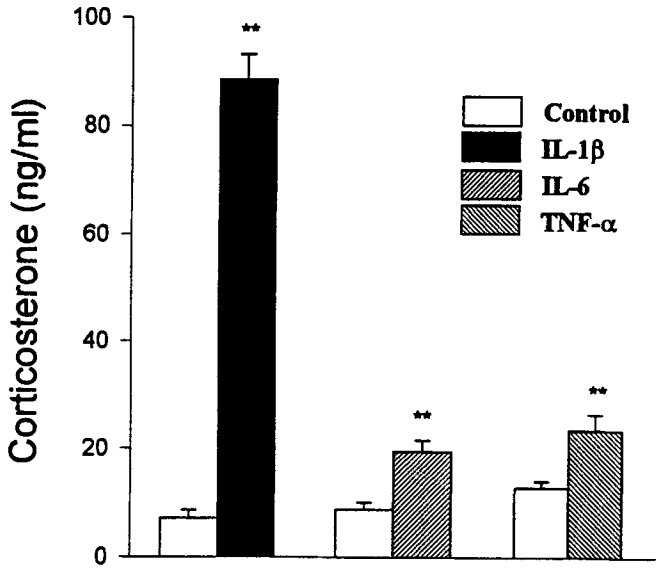

Fig. 3. Effect of IL-1β, IL-6, and TNF-α on the plasma concentration of corticosterone. Eleven day-old rats were intraperitoneally injected with a single dose of IL-1β (6 μg/kg b.wt.), IL-6 (20 μg/kg b.wt.), or TNF-α (20 μg/kg b.wt.) and killed 2 h after the beginning of the experiment. Each bar presents the mean ± SEM of five animals. $^{**}p < 0.01$ vs control; b. wt = body weight.

experiments have been approved by the animal welfare committee of the Liege University.

Cytokines and Other Reagents

Generous gifts of cytokines used in these experiments were: recombinant human interleukin-1β (rIL-1β) from Dr. J. Van Damme, Belgium; rat IL-1β from Dr. Kawashima, Glaxo, Switzerland; recombinant murine TNF-α from Dr. W. Fiers, Belgium; recombinant murine interleukin-6 (rIL-6) from Dr. S. Van Snick, Belgium. 2-Mercaptoethanol was obtained from Merck. RPMI-1640, IMDM, penicillin, and streptomycin were purchased from GIBCO. Tetrahydrochloride spermine, concanavalin (Con A, grade IV) and phorbol myristate acetate (PMA) were bought from Sigma, St. Louis, MO. All other chemicals were of reagent grade. The cytokines used contain a very low concentration of endotoxin.

Spermine Treatment

Spermine (8 μmol), dissolved in 50 μL of NaCl (0.9 %; w/v) (pH 7), was orally administered to 11-d-old rats. Control animals were treated with 50 μL saline (NaCl, 0.9%; pH 7.0). The animals were killed by decapitation 2, 4, 6, and 8 h after spermine administration and trunk blood was collected in tubes containing EDTA and aprotinin (Trasylol). The tubes were then centrifuged at 800*g* for 15 min. Plasma was removed and stored at–70°C until analyses for cytokine concentration were carried out. The control group contained rats treated with saline and killed 0, 2, 4, 6, and 8 h after the beginning of the experiment. The values of cytokine concentrations were pooled and considered as control because there was statistically no difference because of diurnal variation.

Cytokine Treatments

IL-1β (6 μg/kg b. wt.), IL-6 (20 μg/kg b. wt.), or TNF-α (20 μg/kg b. wt.) was ip injected, three times per day, for 3 d, into suckling rats that were 11 d old at the beginning of the experiment. Control rats were treated with 100 μL saline. The result comparison was always made between two groups (control and treated) of five animals coming from the same litter. The animals were killed by decapitation on d 14. The entire small intestine was removed, trimmed of fat and mesentery, and rinsed in ice-cold saline. The intestinal length was measured and divided into two equal segments designed proximal and distal. Intestinal segments were then weighted and homogenized. The homogenates were stored at –70°C until biochemical assays were carried out.

The second group of animals was treated with a single dose of IL-1β (6 μg/kg b. wt.), IL-6 (20 μg/kg b. wt.), or TNF-α (20 μg/kg b. wt.) and was sacrificed 2 h after the beginning of treatment. The blood was collected and treated as described above (*see* Spermine Treatment).

Cell Culture

D10.G4.1, a cloned murine T cell line, was derived from primed AKRJ lymph node T cells *(36)*. A subline of these cells, D10(N4)M (a kind gift of Dr. S. Hopkins, University of Manchester, Great Britain), can be propagated in vitro in the presence of EL4-conditioned medium. Cells were cultured at 37°C/5% CO_2, in RPMI-1640 supplemented with FCS (5%), 2-mercaptoethanol ($5 \cdot 10^{-5}M$), penicillin ($100\ U \cdot mL^{-1}$), streptomycin ($100\ \mu g \cdot mL^{-1}$), and EL4-conditioned medium (10%).

EL-4 cells were grown at 37°C/5% CO_2 in IMDM supplemented with FCS (5%), 2-mercaptoethanol ($5 \cdot 10^{-5}M$), penicillin ($100\ U \cdot mL^{-1}$), and streptomycin ($100\ \mu g \cdot mL^{-1}$). To prepare the conditioned medium, EL-4 cells were harvested by centrifugation and incubated at 10^6 cells/mL in the same medium, together with $10\ \mu g \cdot mL^{-1}$ of concanavalin A (Con A) and $10\ ng \cdot mL^{-1}$ of PMA. After 48 h, the supernatant was harvested and stored at –20°C until use.

A murine hybridoma cell line, 7TD1 (a kind gift from Dr. S. Van Snick, University of Louvain-la-Neuve, Belgium), was used. The cells were grown at 37°C/5% CO_2 in IMDM containing FCS (10%), 2-mercaptoethanol ($5 \cdot 10^{-5}M$), penicillin ($100\ U \cdot mL^{-1}$), streptomycin ($100\ \mu g \cdot mL^{-1}$), and IL-6 ($100\ U \cdot mL^{-1}$).

A fibroblast cell line, WEHI 164 subclone 13 (kindly provided by Dr. Espevik, University of Trondheim, Norway), was grown at 37°C/5% CO_2 in RPMI-1640 containing FCS (10%), penicillin ($100\ U \cdot mL^{-1}$), and streptomycin ($100\ \mu g \cdot mL^{-1}$).

To the authors' knowledge, there were no commercially available antibodies for the rat IL-1β, IL-6, and TNF-α at the beginning of their research. Therefore, they used a

bioassay system (i.e., the best and the more specific system available) as briefly described below complete with all the necessary controls. Afterwards, they also used immunoassay.

IL-6 Bioassay

IL-6 activity in plasma was measured using the IL-6-dependent 7TD1 cell line *(37)*. For the assay, cells were harvested by centrifugation, washed once with medium lacking IL-6. Cells (7000/50 µL) were incubated in the presence of 50 µL serial dilutions of plasma or known concentrations of murine rIL-6. After 72 h incubation at 37°C/5% CO_2, 20 µL of MTT tetrazolium (5 mg · mL^{-1}) were added and the plates were incubated for an additional 4 h. During this period, mitochondrial enzymes in the living cells convert the MTT tetrazolium into dark blue MTT formazan crystals *(38)*. To dissolve these crystals, 100 µL of SDS (10%) in HCl (0.01*M*) were added to each well. The plates were then allowed to stand overnight in the incubator in a humidified atmosphere. On the following day, the plates were read at 540 nm using a microtiter plate (ELISA) reader. The sensitivity of this IL-6 bioassay is equal to 1 pg/mL.

IL-1β Bioassay

The concentration of IL-1β in the plasma was determined using the IL-1β-dependent D10(N4)M cell line *(39)*. The cells were harvested by centrifugation, washed twice with medium lacking the growth factors and then used at 10,000 cells/50 µL flat-bottom well in the presence of 50 µL serial dilution of plasma or known concentrations of recombinant human IL-1β. After 72 h incubation at 37°C/5% CO_2, the cell number was estimated as described for the IL-6 bioassay. The sensitivity of this test is equal to 100 fg/mL.

IL-1β Immunoassay

IL-1β concentration was estimated in serum by using ELISA kit (Biosource International [Camarillo, CA] CYTOscreen™ ELISA kit; rat IL-1β immunoassay kit, no KRC0012).

TNF-α Bioassay

The WEHI 164 subclone 13 cell line was used to measure the concentration of TNF-α in the plasma. The WEHI assay is based on the cytotoxic action of TNF-α on this fibroblast cell *(40)*. In the assay, $3 \cdot 10^4$ cells/50 µL medium were incubated in the presence of actinomycin D (1 µg · mL^{-1}) and 50 µL plasma or different concentrations of murine rTNF-α as standard. After 20 h incubation at 37°C/5% CO_2, cell number was evaluated as described for the IL-6 bioassay. The sensitivity of this test is equal to 100 fg/mL.

TNF-α Immunoassay

TNF-α concentration was estimated in plasma by using ELISA kit (Biosource [Camarillo, CA] CYTOscreen™-US; rat TNF-α immunoassay kit, no KRC3013).

Corticosterone Concentration

The plasma concentration of corticosterone was determined by radioimmunoassay (RIA) using the kit and directions supplied by ICN Biomedicals.

Enzyme Activities, Protein, and DNA Content

Sucrase, maltase, and lactase activities were estimated using the method described by Dalqhvist *(41)*. Protein content was measured according to the method published by Bradford *(42)*, using bovine serum albumin as protein standard. DNA (deoxyribonucleic acid) content was estimated according to the method described by Schneider *(43)*, using calf thymus DNA as standard.

Polyamine Content

Polyamine content in the samples was measured by high-performance liquid chromatography (HPLC) *(44)*. Ten milligrams solid sulfosalicylic acid were added to 300 µL diluted intestinal homogenate. The samples were left overnight. After centrifugation at 3500*g* for 15 min, 200 µL supernatant were taken for derivatization with 500 µL dansylchloride (2 mg · mL^{-1} of acetone) as described previously *(45)*. The dansylated polyamines were separated on a reversed-phase column (Lichrocart RP-18, Merck).

Statistics

All values are expressed as the mean ± SEM. Statistical significance between means was evaluated by the Mann-Whitney nonparametric test for two group comparison or analysis of variance (ANOVA) for multiple group comparison. Results were considered as significant at $p < 5\%$.

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