Cardiovascular and Metabolic Effects of Whole or Fractionated Gram-Negative Bacterial Endotoxin in the Unanesthetized Rhesus Monkey

By Michael J. Reichgott, Kenneth L. Melmon, Ralph P. Forsyth, and Dirk Greineder

ABSTRACT

Rhesus monkeys were infused with endotoxin lipopolysaccharide (LPS) (10 mg/kg [LPS10] or 2.5 mg/kg [LPS2.5]) or with fractions of LPS containing 6.3% lipid (PS1) or 0.5% lipid (PS2) (2.5 mg/kg). Systemic and regional hemodynamics, leukocyte counts, blood gases, pH, and plasma bradykinin concentration were measured. Monkeys receiving LPS10, LPS2.5, or PS1 became hypotensive (mean blood pressure ~37 ± 10 mm Hg) and had decreased peripheral vascular resistance (~10% to ~24% of the base line), compensated metabolic acidosis, and elevated plasma bradykinin concentrations (~4 ± 6 ng/ml) 2 hours after infusion. Vasodilation occurred in coronary, hepatic, and splanchnic vasculature; vasoconstriction occurred in the spleen. Cardiac output was diverted from muscle to viscera. Monkeys receiving PS2 were normotensive with elevated peripheral vascular resistance (~46%) and no measurable plasma bradykinin concentration. By 6 hours, marked elevation of peripheral vascular resistance developed in monkeys given LPS10 (~113%) and LPS2.5 (~57%). Monkeys receiving PS1 returned to base-line values, but monkeys receiving PS2 remained unchanged. Leukopenia (~50% to ~65%) was persistent only in monkeys receiving LPS or PS1. Toxicity of LPS apparently depends on the lipid portions of the molecule. Vasodilation and bradykinin generation are correlated with persistent granuloeytopenia. Late toxicity may be independent of early cardiovascular events.

KEY WORDS

lipopolysaccharide
bradykinin
microspheres
cardiac output
polysaccharides
granuloeytopenia
regional blood flow

Gram-negative bacterial endotoxin, a complex lipopolysaccharide, causes species-specific effects (1). Primates, both human and subhuman, develop vasodilation, granuloeytopenia, and elevated plasma concentrations of bradykinin as the initial response to endotoxemia (2-4). Although granuloeytopenia is common, vasodilation does not occur in other species exposed to endotoxin (1), and bradykinin does not participate in the endotoxin-shock syndrome in these animals (5-6).

Leukocyte kinin-generating capabilities differ between primates and other species (7-9), but plasma kinin-generating systems are similar (9-10). Leukocyte interactions with endotoxin depend on lipid portions of the bacterial preparation (11-12), although endotoxin-plasma interactions require only bacterial polysaccharide antigens (12).

Therefore, the interaction of leukocytes with lipid portions of endotoxin may result in the generation of vasoactive substances which mediate the cardiovascular events of endotoxemia. Bradykinin may be an important mediator in the primate (3, 13). To test this hypothesis, lipid-containing and lipid-free preparations of bacterial endotoxin were infused into unanesthetized Rhesus monkeys. The results confirmed the importance of lipids for both the generation of bradykinin and the development of hemodynamic effects. The results also supported the hypothesis that interaction of endotoxin with leukocytes is the primary pathogenetic mechanism.
However, the late-phase cardiovascular and metabolic toxicity of endotoxin may not develop as a direct progression from the early-phase cardiovascular effects.

**Methods**

Studies were performed in nine Rhesus monkeys (Macaca mulatta) of either sex weighing 4.3-7.4 kg. The monkeys were anesthetized with sodium pentobarbital (25-30 mg/kg, iv.), and polyvinyl catheters were inserted into the inferior vena cava and the abdominal aorta via the left common iliac vessels. During a separate operation, using fluoroscopic observation, a third catheter was passed retrograde through the left common carotid artery into the left ventricle. The catheters were guided through prepared subcutaneous tracts and exteriorized at the level of the umbilicus.

After recovering from anesthesia, the monkeys were placed into restraining chairs modified to allow tilting inside isolation booths. The catheter lines were brought inside isolation booths. The catheter lines were brought to the outside of the booths and were kept patent by continuous infusion of lightly heparinized (5 USP units/ml) 0.9% sodium chloride at 1 mg/hour. All experiments were performed a minimum of 7-10 days postoperatively to avoid the cardiovascular effects of recent surgical procedures and anesthesia. Monkeys have been shown to remain stable for several months under these conditions (14). Details of this preparation have been reported previously (15).

Measurements of arterial and central venous blood pressures were obtained continuously using Statham P23Cb strain gauges placed at the midthoracic level. Mean blood pressure was derived electronically. A cardiac output coupler was used to measure heart rate. All recordings were made on a Beckman type R recorder. Blood samples were obtained from the arterial catheter at intervals; pH, Po2, and Pco2 were measured using Radiometer microelectrodes and corrected to 38°C. Hematocrit was measured using a microtechnique, and complete and differential leukocyte counts were made.

Cardiac output was determined by the indicator-dilution technique using indocyanine green dye. Injections of 1 ml of dye (0.5 mg/ml) were made into the left ventricle using a spring-loaded constant-volume syringe. Blood was withdrawn from the arterial catheter at intervals; pH, Po2, and Pco2 were measured using Radiometer microelectrodes and corrected to 38°C. Hematocrit was measured using a microtechnique, and complete and differential leukocyte counts were made.

REGIONAL DISTRIBUTION OF CARDIAC OUTPUT

Measurements of the regional distribution of cardiac output were made using the method of Rudolph and Heymann (17). After determination of total cardiac output, one of five differently labeled batches (125I, 141Ce, 51Cr, 85Sr, or 99Nb) of radioactive microspheres (50μ in diameter) was injected into the left ventricle. These microspheres mix with the blood in the left ventricle and are distributed with the cardiac output. They become impacted in arterioles and remain in the tissues.

After the monkeys were killed by intravenous injection of sodium pentobarbital, organs and tissues were dissected and weighed. Samples of the organs and tissues were placed into glass vials and were counted in a gamma scintillation counter (Nuclear Chicago).

Pulse-height analysis of the radioactive energy spectra present in each organ allows determination of the amount of radioactivity due to each radionuclide. The radioactivity due to each nuclide in the whole monkey was determined by summation of the calculated radioactivity of all the organs and tissues. The amount of radioactivity due to each nuclide within each organ compared with whole-body radioactivity due to that nuclide defines the fractional distribution of cardiac output to that organ at the moment that that nuclide was injected. When this fraction is multiplied by the total cardiac output, the actual blood flow to each organ and the regional vascular resistance at the time of microsphere injection can be calculated. Studies documenting the validity of this method in the primate have been reported (18).

ENDOTOXIN AND FRACTIONS

The endotoxin preparations used in these studies were produced and characterized by Greineder (19). Phenol-water extracts of bacterial cell walls were incubated with ribonuclease. After dialysis the "crude" lipopolysaccharide was centrifuged at 185,000 g for 90 minutes. The pellet consisted of "pure" lipopolysaccharide (LPS), and portions of this material were used in the present experiments. Subfractionation of LPS was performed by acid hydrolysis using 0.02N acetic acid at 100°C. Centrifugation produced a precipitate. Lipid A, 40-50% yield by weight. The supernatant fluid was chromatographed on charcoal. A polysaccharide fraction (PS1) containing 6.3% of the total fatty acids present in LPS at yields of 20-25% by weight was eluted with water. A second elution using 0.05N H2SO4 in 50% ethanol produced fraction PS2. This polysaccharide, obtained in approximately 20% yield, contained less than 0.5% of the original lipid and was considered to be lipid free. Both PS1 and PS2 have molecular weights greater than 100,000 (chromatography on Bio-Gel P100 equilibrated with 0.1x sodium chloride and 0.01× Tris buffer, pH 7.5).

Small amounts of these fractions, lyophilized at the time of preparation, were frozen until used. They were prepared for intravenous administration by suspension in sterile 0.9% sodium chloride at an initial concentration of 1 mg/ml followed by shaking for 24 hours at room temperature. The resultant suspensions were centrifuged at 1,000 g in a Sorvall RC2B centrifuge. Supernatant fluids were saved and refrigerated until they were used. Samples were examined at 500 nm in a...
The effects of LPS and the PS fractions are outlined in Table 1; only mean values are presented since the groups were small.

LPS sub caused a transient increase in peripheral vascular resistance followed by progressive vasodilation. By 2 hours resistance had fallen 10% below baseline, and mean systemic arterial blood pressure had declined 41 mm Hg. LPS sub caused progressive vasodilation without initial vasoconstriction. At 2 hours, resistance had fallen 24% and mean systemic arterial blood pressure was 27 mm Hg below baseline values. At 6 hours both LPS sub and LPS 2.5 groups demonstrated marked vasoconstriction. Total peripheral vascular resistance had increased 113% and 57%, respectively. Arterial hypotension persisted in both of these groups.

Monkeys given PS sub (6.3% lipid) developed progressive vasodilation; peripheral vascular resistance decreased 24% and blood pressure had fallen 43 mm Hg at 2 hours. Peripheral vascular resistance and mean blood pressures both returned to base line by 6 hours. Conversely, monkeys given PS sub (0.5% lipid) had an early 46% rise in peripheral vascular resistance. Blood pressure fell slightly and cardiac output decreased approximately 25%. By 2 hours, these monkeys had reached a plateau of both pressure and resistance. A similar pattern of nonspecific resistance and pressure changes has been noted previously in monkeys tilted and subjected to microsphere infusions only (3, 18). PS sub may actually have increased these nonspecific effects on cardiac output and peripheral resistance, but the group size was too small to allow this conclusion.

### Regional Hemodynamic Effects of Endotoxin Fractions

The effects of LPS or PS fractions on distribution of cardiac output and calculated vascular resistance measured at 2 hours, are presented in Tables 2 and 3. Kinin generation and systemic hemodynamics were similar in the seven monkeys receiving lipid-containing infusions; therefore these monkeys were grouped together for analysis.

At 2 hours, lipid-exposed monkeys (LPS 10, LPS 2.5, and PS sub) had significant changes in the patterns of regional distribution of cardiac output compared with base-line values (Table 2). Fractional blood flow had increased to the heart, the liver (via the hepatic artery), and the gut. Flow was decreased in the spleen and the skeletal muscle. The lipid-free group (PS sub) had an increased fraction of cardiac output to the kidney and the spleen (probably at the expense of muscle).

Significant changes in tissue resistance were also noted (Table 3) at 2 hours in the lipid-exposed group.
monkeys. Resistance was decreased in the heart, the total liver (combination of both hepatic artery and portal vein), the gut, and the pancreas. There was significant vasoconstriction in the spleen. An almost identical pattern of resistance change in response to endotoxin has previously been reported in the primate (2). Monkeys receiving PS2 had significantly increased resistance only in the gut, but there is a suggestion that some increase in resistance had

Table 2

<table>
<thead>
<tr>
<th>Organ</th>
<th>Base line All monkeys (0)</th>
<th>Lipid-containing (7)</th>
<th>Lipid-free (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>5.4 ± 0.7</td>
<td>9.2 ± 2.4</td>
<td>7.5 ± 3.5</td>
</tr>
<tr>
<td>Brain</td>
<td>5.2 ± 1.3</td>
<td>4.7 ± 0.7</td>
<td>6.2 ± 1.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>15.3 ± 3.0</td>
<td>14.3 ± 2.9</td>
<td>19.3 ± 1.8*</td>
</tr>
<tr>
<td>Liver</td>
<td>19.8 ± 3.0</td>
<td>26.2 ± 5.0*</td>
<td>19.0 ± 2.1</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>6.6 ± 3.3</td>
<td>11.7 ± 4.8*</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>Portal vein</td>
<td>14.3 ± 3.0</td>
<td>14.4 ± 0.7</td>
<td>13.0 ± 3.0</td>
</tr>
<tr>
<td>Gut</td>
<td>7.8 ± 1.5</td>
<td>11.0 ± 1.4*</td>
<td>7.0 ± 1.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.1 ± 0.8</td>
<td>0.7 ± 0.5*</td>
<td>3.1 ± 0.01*</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.7 ± 0.6</td>
<td>1.4 ± 0.4</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>Skin</td>
<td>5.7 ± 1.7</td>
<td>4.3 ± 1.4</td>
<td>6.5 ± 1.6</td>
</tr>
<tr>
<td>Bone</td>
<td>4.1 ± 1.6</td>
<td>3.3 ± 0.9</td>
<td>4.9 ± 0.6</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>20.1 ± 5.9</td>
<td>17.2 ± 3.5*</td>
<td>18.4 ± 2.4</td>
</tr>
</tbody>
</table>

Number of monkeys exposed is given in parentheses. *Differs from base line, \( P < 0.05 \) derived from Student's t-test.

Table 3

<table>
<thead>
<tr>
<th>Organ</th>
<th>Base line All monkeys (0)</th>
<th>Lipid-exposed (7)</th>
<th>Lipid-free (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.7 ± 0.6</td>
<td>0.8 ± 0.3*</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Brain</td>
<td>9.1 ± 2.9</td>
<td>7.9 ± 3.1</td>
<td>10.4 ± 1.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Liver</td>
<td>4.1 ± 1.3</td>
<td>2.6 ± 1.1*</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>15.0 ± 7.1</td>
<td>13.4 ± 8.3</td>
<td>16.0 ± 4.0</td>
</tr>
<tr>
<td>Portal vein</td>
<td>8.8 ± 2.4</td>
<td>6.7 ± 2.0</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td>Gut</td>
<td>10.2 ± 2.4</td>
<td>5.1 ± 2.9*</td>
<td>14.7 ± 1.6*</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.5 ± 1.1</td>
<td>7.8 ± 4.8*</td>
<td>3.5 ± 2.6</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.5 ± 0.7</td>
<td>1.4 ± 0.4*</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Skin</td>
<td>41.6 ± 18.3</td>
<td>48.4 ± 20.8</td>
<td>35.5 ± 11.6</td>
</tr>
<tr>
<td>Bone</td>
<td>68.2 ± 34.9</td>
<td>76.5 ± 20.0</td>
<td>72.1 ± 29.0</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>39.9 ± 15.8</td>
<td>50.0 ± 17.6</td>
<td>59.7 ± 15.5</td>
</tr>
</tbody>
</table>

Number of monkeys exposed is given in parentheses. *Differs from base line, \( P < 0.05 \) determined by Student's t-test.
occurred in other vascular beds (primarily hepatic and muscle). The variability of systemic vascular resistance between groups at 6 hours (Table 1) was mirrored in the regional hemodynamic patterns. Monkeys receiving LPS10 had increased resistance in almost all vascular beds, notably highest in the spleen and the pancreas. Only the hepatic artery remained dilated. Monkeys receiving LPS2.5 also had increased vascular resistance in all beds except the hepatic artery, but the levels were much lower than those in the LPS10 group. Monkeys receiving PS1 (6.3% lipid) had recovered baseline resistance values except in the splenic vascular bed where resistance was quite high. Monkeys receiving PS2 did not change between 2 and 6 hours. The small group sizes preclude further evaluation of these late-phase vascular resistance changes.

METABOLIC EFFECTS OF ENDOTOXIN FRACTIONS

All monkeys tended to hyperventilate, as indicated by the decreases in arterial PO2 (Table 1), but alkalosis developed only in those monkeys given PS2. The relative constancy of pH in the lipoid-exposed monkeys suggests simultaneous development of metabolic acidosis. Transient leukopenia occurred in all monkeys, but it persisted without recovery at 2 hours only in lipid-exposed monkeys. Leukopenia was due primarily to granulocytopenia (Table 1).

BRADYKININ GENERATION

Monkeys receiving LPS10, LPS2.5, and PS1 developed high concentrations of bradykinin in their arterial blood; however, monkeys receiving PS2 did not (Table 4). The highest measured concentrations of bradykinin occurred immediately prior to the nadir of peripheral vascular resistance. Bradykinin generation occurred only in those groups of monkeys exhibiting prolonged granulocytopenia (Table 1).

<table>
<thead>
<tr>
<th>Time of observation</th>
<th>Exposed (LPS, PS)</th>
<th>Free</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base line</td>
<td>0 (7)</td>
<td>0 (2)</td>
<td></td>
</tr>
<tr>
<td>15 minutes-2 hours (grouped)</td>
<td>14 ± 6 (21) 0 (2) &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes</td>
<td>12 ± 6 (7) 0 (2) &lt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>16 ± 6 (7) 0 (2) &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hours</td>
<td>14 ± 6 (7) 0 (2) &lt; 0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Monkeys receiving lipid-containing endotoxin fractions were grouped together.
†Below sensitivity of method.

Discussion

Although extensively studied, the structure-activity relationships of gram-negative endotoxin lipopolysaccharide remain incompletely defined (22). The polysaccharide is the major antigenic determinant of the macromolecule and corresponds to the somatic O-antigen of the bacterium (23). The lipid-containing portion of the molecule is responsible for the cellular toxicity of endotoxin (22-24). Preparations with only small amounts of lipid may retain toxic potential (25), and lipid-free preparations are antigenic but nontoxic (26). Whether cellular toxicity is linked to cardiovascular changes is not known. Endotoxin neither directly affects vascular smooth muscle function (27) nor depresses cardiac function (28, 29). However, endotoxin can interact with both cellular systems and noncellular plasma enzymes to release a variety of active substances such as leukocyte pyrogen (23, 30), bradykinin (5, 9-13, 31), and lysosomal enzymes (11-12) and to activate both the clotting (31) and immune mechanisms (32).

The antigenic polysaccharide portion of endotoxin, in the presence of 19S macroglobulin antibody and complement, releases bradykinin and depletes kininogen in a cell-free plasma system (10, 12). The interaction of endotoxin with leukocytes has the characteristics of phagocytosis with increased RNA production, increased lactate and carbon dioxide production, and release of lysosomal enzymes into the suspending medium (11). This interaction is dependent on available complement but not on 19S antibody (11) and requires lipid-containing high molecular weight endotoxin (12).

The experiments reported in this paper indicate that there is in the primate an in vivo relationship between the presence of lipid and the development of the early cardiovascular changes of endotoxemia. In these experiments, whole endotoxin (LPS10) reproduced all the systemic, regional, and metabolic characteristics of endotoxemia produced previously by endotoxins derived from other bacterial strains except that total peripheral vascular resistance during the acute shock period did not fall to the same extent as previously found (2, 3). Slow endotoxin infusion is associated with vasodilation in primates, but rapid endotoxin injection results in acute vasoconstriction (33). This vasoconstriction is probably a nonspecific reflex event caused by the particulate suspension being injected. Our present preparations, particularly the higher concentrations of LPS (LPS10) may be demonstrating this.
particulate effect. This observation is partly confirmed by the more characteristic vasodilation that occurred in monkeys infused with smaller doses of whole endotoxin (LPS₂₀). Greater dilution of LPS would limit the particulate effect, making early vasodilation more evident. Active vasodilation also occurred in monkeys given PS₁, but monkeys receiving PS₂ became vasoconstricted. The obvious common denominator for early vasodilation was the presence of a minimum amount of lipid material.

In man and the subhuman primate, endotoxin infusion results in generation of large amounts of bradykinin (3, 4). The early, sustained decreases in peripheral vascular resistance which are a unique pattern of endotoxin shock in these species correlate closely in time with kinin production but not with identifiable amines (3, 4). In other species such as dog, cat, and rabbit, early vasodilation is either absent or minimal (1). Shah et al. (6) have demonstrated only very low levels of circulating bradykinin in the dog exposed to endotoxin. We have not been able to demonstrate bradykinin release following endotoxin infusion into cats, and the rabbit produces only small amounts of bradykinin by interaction of endotoxin with the plasma system (5, 9) but lacks rapidly acting white cell cytoplasmic kallikrein or kallikrein activator (7, 8), which probably is most important for significant peptide production (7). Bradykinin does not contribute to septic shock in this species and vasodilation does not occur (5, 34).

In the present experiments, the vasodilation and the distribution of cardiac output, characteristic of endotoxemia, developed only in those monkeys in which kinin generation occurred. This association does not prove that bradykinin is the mediator of the cardiovascular changes, but it does reconfirm the close relationship between kinin generation and cardiovascular effects of endotoxin in the primate.

Prolonged granulocytopenia, kinin generation, and the presence of larger amounts of lipid in the infused endotoxin material are also correlated. Leukopenia occurred in all experimental groups; in the monkeys receiving lipid-free PS₂, it was of short duration, was not due to granulocytopenia, and was unassociated with any measurable kinin generation. Vasodilation occurred in the three groups given material with larger amounts of lipid; leukopenia was prolonged for at least 2 hours, was primarily due to granulocytopenia, and was associated with kinin generation and with vasodilation. An activation period has been clearly demonstrated in the endotoxin-granulocyte interaction resulting in pyrogen production (34). Therefore, the association of kinin generation and vasodilation only with prolonged granulocytopenia is consistent with the concept that similar activation of kinin-generating mechanisms may be required.

These early-phase results suggest that the generation of bradykinin and the development of vasodilation are dependent on the administration only of a minimum sufficient amount of lipid material. LPS₁₀ monkeys received approximately 5 ng/kg of lipid and PS₁ monkeys received only 0.075 ng/kg. Despite this wide difference in dose the early effects of these fractions were quite similar. PS₂, although essentially lipid-free, is not devoid of effects on the cardiovascular responses of the whole monkey or on leukocytes. Nonetheless, the very different pattern of cardiovascular events, the transient leukopenia, and the lack of kinin generation only serve to underscore the importance of the lipid portions of the molecule to these early effects. Extensive analysis of these PS fractions (19) has shown that one significant difference between PS₁ and PS₂ is in the content of lipid material.

Extension of these observations to the late-phase (6 hours) results obtained in these experiments is limited by the small groups which could be studied. However, these preliminary observations suggest that the systemic and regional hemodynamic events at this later time are more closely lipid dose-related than are the early-phase events. Conversely, they seem to be independent of the early-phase cardiovascular instability and release of active mediator. This dichotomy of effect may have bearing on the question of the transition to irreversible shock.

Apparent metabolic acidosis was also lipid-dependent.

The phenomena reported in this paper indicate that the specific cellular toxicity of endotoxin resides in the lipid portions of the molecule. The cardiovascular events of early endotoxemia are associated with kinin generation and granulocytopenia and may therefore be, in part, consequences of such LPS toxicity on the granulocyte. The later toxic effects of endotoxin also appear to be lipid related but may be due to an entirely different mechanism.

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