Effects of Bradykinin and Autonomic Nervous System Inhibition on Systemic and Regional Hemodynamics in the Unanesthetized Rhesus Monkey

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

ABSTRACT

Studies were performed in unanesthetized monkeys to determine if bradykinin infusions reproduce the circulatory events of early endotoxemia. Kinin infusions before and during autonomic ganglionic blockade with trimethaphan were significantly correlated (P<0.05) with decreases in mean arterial pressure. Kinin infusion at 15-18 µg/kg min⁻¹ produced 26 mm Hg fall in mean blood pressure at 3 min, due to fall in total peripheral resistance of 14 mm Hg/liter min⁻¹. Heart rate rose 23 beats/min. After 10 minutes of infusion, peripheral resistance had returned to base line, blood pressure remained low due to fall in cardiac output of 0.53 liters/min (P<0.01). Ganglionic blockade prevented recovery of resistance. Plasma bradykinin levels at 3 and 10 minutes were 14 and 15 ng/ml, respectively. Regional and systemic hemodynamic effects of kinin (15–18 µg/kg min⁻¹) were determined in 10 monkeys. After 10 minutes of infusion, bradykinin produced systemic effects. Regional flow measurement (by radioactive microsphere technique) demonstrated a pattern similar to that seen during hemorrhage. Ganglionic blockade lowered mean arterial pressure 33 mm Hg by generalized vasodilatation. Kinin infusion then resulted in further vasodilatation and fall in blood pressure of 12 mm Hg, and cardiac output of 0.74 liters/min. Regional flow distribution during combined infusion was similar to that seen during early endotoxemia.

KEY WORDS microspheres organ blood flow endotoxin shock peripheral vascular resistance sympathetic nervous system vasodilators cardiac output blood pressure hemodynamics

The pathophysiologic mechanisms of the cardiovascular changes of septic shock or experimental endotoxemia are unknown. Many patients with early sepsis demonstrate a "hyperdynamic shock state" characterized by persistently low peripheral vascular resistance and a relatively normal or even elevated cardiac output (1–6). Infusion of well-dissolved endotoxin has reproduced this syndrome in the subhuman primate (7). This unusual pattern of systemic cardiovascular response has led to a search for vasodilators as trigger substances or mediators of these effects (8).

Elevated levels of bradykinin have been found both in experimental endotoxemia in the monkey (9) and spontaneous sepsis in man (10–12). Changes in plasma bradykinin levels in vivo during experimental endotoxemia in the primate correlate with the appearance of the shock state (9), and endotoxin can activate at least two kinin-generating systems in vitro (13). Bradykinin has been implicated, therefore, as one possible mediator of endotoxin shock in the primate.

The experiments reported here were designed to study the effects of bradykinin on the circulation of the unanesthetized primate and to test whether bradykinin could mimic...
the specific regional blood flow changes produced by endotoxin (7). An alternative mechanism for the sustained decrease of peripheral resistance during early endotoxemia is lack of effective reflex cardiovascular response to hypotension (14). To test this possibility, we also examined the regional and systemic hemodynamic changes occurring during autonomic ganglionic blockade before and during bradykinin infusion. A pattern of systemic and regional cardiovascular changes similar to that seen in the early endotoxin shock state was reproduced by bradykinin infusion only when reflex responses to hypotension had been inhibited by autonomic nervous system blockade.

**Methods**

Rhesus monkeys of either sex weighing 4.4–7.8 kg were studied. The animals were anesthetized with pentobarbital sodium, and polyvinyl catheters were inserted into the inferior vena cava and abdominal aorta below the renal arteries via the common iliac vessels. At a separate operation, a third catheter was inserted retrograde through the left common carotid artery into the left ventricle with fluoroscopic control. The catheters were exteriorized at the level of the umbilicus.

After recovering from anesthesia, the animals were placed into restraining chairs modified to allow tilting inside isolation booths, and the catheter lines were brought to the outside of the booths. The catheters were kept patent by continuous infusion of lightly heparinized (5 USP units/ml) 0.9% NaCl at 1 ml/hour. Details of this preparation have been previously reported (15). All experiments were performed at least 7–10 days postoperatively to avoid effects of recent surgical procedures and anesthesia. The animals were tilted at least 1 hour before the experiments were begun, to eliminate orthostatic hemodynamic effects.

Arterial and central venous pressures and heart rate were measured with Statham 23Gb strain gauges adjusted to the midthoracic level and a cardiotachometer coupler. Recordings were made on a Beckman type R recorder.

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 and blood was withdrawn from the arterial catheter (length 75 cm, internal volume 1 ml) at constant rate (10 ml/min) under sterile conditions. The dye curve was recorded with a Waters XP 302 densitometer, and the blood was immediately returned to the animal. The reported values are the average of at least two technically adequate dye curves. Total peripheral resistance was calculated as arterial-venous blood pressure difference divided by cardiac output (mm Hg/liter min⁻¹).

Blood samples were obtained intermittently from the arterial catheter. PaO₂, PaCO₂, and pH were measured with Radiometer microelectrodes and corrected to 38°C. Hematocrit was measured by the microtechnique.

Bradykinin trisuccinate trihydrate (mol wt 1594.41) (Cal Biochem Co.) was prepared by dilution in 0.9% NaCl to 1 mg/ml; 2-ml samples were frozen and a new sample thawed for each day's experiment. Before infusion, the bradykinin was further diluted in saline. Infusions were made intravenously using a Harvard constant-speed infusion pump. The bradykinin was diluted, stored, and infused using sterile plastic equipment.

**DOSE-RESPONSE STUDIES**

Bradykinin was infused intravenously in doses of 4–37 μg/kg min⁻¹ in six animals while continuous records of blood pressure and heart rate were obtained. Infusions lasted 3 minutes with 10 minutes between each infusion. A total of 64 such infusions were performed, and each animal received at least 4. The size of the doses was randomized, and animals received a maximum of 3 mg of bradykinin in any single day.

In five tilted animals, the bradykinin dose-response relationship also was determined during ganglionic blockade. Autonomic ganglionic blockade was established by intravenous administration of trimethaphan camsylate, 1 mg/ml, at a rate adjusted so that the mean arterial pressure would fall approximately 40 mm Hg without associated increase in heart rate. The infusion was then maintained at this constant rate, and bradykinin was administered simultaneously through the other arm of a Y-tube. A total of 39 bradykinin infusions of 4 to 30 μg/kg min⁻¹ were performed. Each animal received at least five randomly ordered doses of kinin as previously described.

**SYSTEMIC EFFECTS OF KININ INFUSION**

Bradykinin (15–18 μg/kg min⁻¹) was infused intravenously for 10 minutes 11 times in seven monkeys to characterize the systemic hemodynamic effects of the peptide. The four animals receiving more than one infusion were rested for at least 3 days between experimental studies, although all measurements had returned to base line within 6 hours of the completion of the first infusion. In each animal, a single value for blood pressure or heart rate represents the average of at least five observations under stable conditions. Blood pressures and heart rate were measured.
continuously, and cardiac output determined 3 and 10 minutes after the start of the infusion.

**PLASMA BRADYKININ DETERMINATION**

Plasma bradykinin levels were determined at 3 and 10 minutes during infusions of kinin at 15–18 µg/kg min⁻¹, and 20 minutes after completion of the infusion. Five ml of arterial blood was withdrawn into 10 ml of 0.5M perchloric acid. After adjustment of pH to 5.4, bradykinin was separated by passage over IRC-50 (H⁺ form) resin. Peptide was eluted from the resin with 0.5M NaCl-0.5M ammonium formate buffer as previously described (9). Peptide was then assayed by radioimmunoassay by a minor modification of the method of Talamo et al. (16).

**REGIONAL HEMODYNAMIC STUDIES**

Regional distribution of cardiac output was studied in ten monkeys before and after the establishment of autonomic ganglionic blockade. Following an initial base-line period, bradykinin was infused 15–18 µg/kg min⁻¹ for 10 minutes and measurements made. The animals were then rested for 6 hours or longer and repeated base-line measurements obtained. Ganglionic blockade was then established with trimethaphan as previously described and measurements were repeated. Finally, bradykinin was infused as before, with blockade maintained by constant trimethaphan infusion, and another set of measurements was obtained. Measurements included systemic arterial blood pressure, cardiac output, heart rate, arterial hematocrit, PaO₂, PaCO₂ and arterial pH. Total peripheral resistance was calculated as previously described.

Regional distribution of flow was determined by the method of Rudolph and Heymann (17) by infusion of one of five differently labeled batches of radioactive microspheres (50 µm diameter) into the left ventricle. The radioactive labels were 125I, 141Ce, 61Cr, 86Sr, or 99mNb. The labeled microspheres mix with the blood in the left ventricle and are distributed to end organs in direct proportion to the blood flow to each organ. The amount of radioactivity of each isotope in an organ (determined with a Nuclear Chicago gamma scintillation counter and a pulse height analyzer) relative to the total radioactivity counted in the animal, represents the fraction of cardiac output received by that organ at the time of the microsphere injection. The fraction to each organ at the time of each isotope infusion, times the cardiac output as determined by indicator dilution, gives the absolute flow to each organ. Regional vascular resistance was calculated from the formula \( R = (P_a - P_v)/flow \). Details of the analysis of the energy spectrum in each organ, allowing calculation of flow related to each nuclide, have been previously reported (17).

Studies of the validity and reliability of this method (18) indicate that good mixing of microspheres is obtained in the left ventricle and that distribution of spheres to organs is a reliable measurement of fractional distribution of cardiac output provided the organ receives more than 1% of the total cardiac output. Both the systemic and regional hemodynamic changes were compared to changes in a control group of seven tilted, undisturbed monkeys infused with four batches of microspheres (18).

The animals were killed by injection of pentobarbital sodium, and at autopsy 23 organs and tissues were weighed, placed into glass vials, and counted in the gamma scintillation counter.

Statistical analyses were performed by the two-tailed t-test for independent groups for systemic measurements, and the nonparametric Mann-Whitney U-test for the regional circulatory measurements.

**Results**

**DOSE-RESPONSE RELATIONSHIPS**

Figure 1 illustrates the dose-response relationship between bradykinin and mean arterial blood pressure before and after ganglionic blockade. Approximately 30 seconds after the start of bradykinin infusion, blood pressure fell sharply. It then stabilized and reported values were obtained during this stable period. Before blockade, in the range of doses examined, a significant Pearson product-moment correlation was found \( r = -0.711; P < .001 \). The formula for the regression line was \( Y = -7.56 - 0.937X \). Ganglionic blockade resulted in a fall of mean blood pressure of 42 ± 8.6 mm Hg \( (P < .001) \). Simultaneous infusion of bradykinin during blockade produced a further fall of Pa \( (r = -0.894; P < .05) \); the regression line's formula was \( Y = -48.2 - 0.227X \).

As the total amount of bradykinin infused into an animal increased, significant changes occurred in all hemodynamic measurements. The earliest changes were noted in heart rate and total peripheral vascular resistance, both of which rose rapidly. Cardiac output fell simultaneously with these changes, and rises in hematocrit occurred more slowly. The baseline value of blood pressure was stable until more than 2 mg of kinin had been infused.

The effects of identical doses of kinin given before and after the midpoint of the total dose...
Dose-response relationship of bradykinin and fall in mean arterial blood pressure before and during autonomic ganglionic blockade. Mean and SE are indicated at each dose level. Numbers in parentheses refer to the number of observations at each dose.

(1200 μg) were compared and did not differ. Therefore all doses were analyzed without regard to the time of their administration.

**SYSTEMIC EFFECTS OF BRADYKININ INFUSION**

The effects of kinin infusion at a dose of 15-18 μg/kg min⁻¹ on blood pressure, heart rate, cardiac output, and total peripheral resistance after 3 and 10 minutes of infusion are shown in Table 1. Both pressure and resistance were significantly decreased by 3 minutes; cardiac output was unchanged, and heart rate was increased. After 10 minutes of infusion, the cardiac output had fallen significantly and resistance had returned to base line. Blood pressures remained significantly decreased.

**DOCUMENTATION OF PLASMA BRADYKININ LEVELS**

In the four monkeys tested, the mean arterial blood levels of measured bradykinin after 3 minutes of infusion was 14 ± 4 ng/ml. At the end of the infusion (10-minute sample) the mean blood level was 15 ± 3 ng/ml, indicating that stable levels of the plasma kinin had been achieved by 3 minutes of infusion. Twenty minutes after the end of the infusion, blood levels were found to be 1 ± 1 ng/ml, identical to levels before infusion.

**REGIONAL DISTRIBUTION OF CARDIAC OUTPUT**

The systemic hemodynamic measurements obtained during these experiments are presented in Table 2. Effects of 10-minute infusions of bradykinin (15-18 μg/kg min⁻¹) on cardiovascular variables are essentially the same as those reported at 10 minutes in the previous group (Table 1).

Base-line values after rest did not differ significantly from initial values. Blood pressure, cardiac output, and total peripheral resistance all fell during autonomic blockade. Bradykinin infusion during maintained ganglionic blockade resulted in further fall in all these measurements. After 10 minutes of

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<table>
<thead>
<tr>
<th>Measurement</th>
<th>Base line</th>
<th>Bradykinin Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>102 ± 6</td>
<td>76 ± 14*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>176 ± 26</td>
<td>199 ± 22</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>1.99 ± 0.73</td>
<td>2.03 ± 0.73</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg/L min⁻¹)</td>
<td>54 ± 15</td>
<td>40 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SD of 11 observations in seven monkeys.

*P < 0.01, paired t-test, infusion period compared to base line.
### Table 2
Systemic Effects of Bradykinin Infusion (15-18 μg/kg min⁻¹) for Ten Minutes in Ten Monkeys before and after Ganglionic Blockade

<table>
<thead>
<tr>
<th></th>
<th>First base line</th>
<th>Bradykinin infusion</th>
<th>Second base line</th>
<th>Ganglionic blockade</th>
<th>Bradykinin + Ganglionic blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic arterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td>128 ± 13</td>
<td>102 ± 12*</td>
<td>131 ± 13</td>
<td>90 ± 15*</td>
<td>76 ± 12*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>97 ± 14</td>
<td>75 ± 11*</td>
<td>99 ± 13</td>
<td>66 ± 15</td>
<td>54 ± 9*</td>
</tr>
<tr>
<td>Diastolic arterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td>68 ± 13</td>
<td>51 ± 11*</td>
<td>71 ± 11</td>
<td>44 ± 15*</td>
<td>35 ± 9*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>184 ± 22</td>
<td>196 ± 25</td>
<td>185 ± 32</td>
<td>176 ± 22</td>
<td>175 ± 20</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>2.44 ± 0.7</td>
<td>1.75 ± 0.9*</td>
<td>2.20 ± 0.8</td>
<td>1.62 ± 0.5</td>
<td>1.46 ± 0.5</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg/L min⁻¹)</td>
<td>42 ± 10</td>
<td>49 ± 15</td>
<td>49 ± 13</td>
<td>43 ± 14 †</td>
<td>41 ± 10 †</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>31 ± 1</td>
<td>32 ± 2</td>
<td>32 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± sd.  
*P < 0.01, t-test, as above; †Electronically averaged; †P < 0.05 (t-test) compared to changes in seven control monkeys.

infusion, peripheral resistance was still decreased, in contrast to the effect of kinin infusion without simultaneous blockade. Hematocrit was also lower. Blood gases and pH were stable throughout the study (pH 7.45 ± 0.02; PaO₂ = 106 ± 4.3; PaCO₂ = 35.1 ± 1.4).

The effects of bradykinin alone, autonomic blockade alone, and the combined effects on distribution of cardiac output, blood flow, and resistance in the organs and systems showing significant changes are presented in Table 3.

#### Discussion

If vasoactive substances mediate the cardiovascular changes seen in early endotoxemia, they should satisfy several criteria: (1) endotoxin must be capable of causing release or generation of the active substances; (2) the vasoactive agents must be present before or during the cardiovascular events; (3) infusion of the vasoactive agents must produce the specific cardiovascular effects; and (4) inhibition of the action or formation of these vasoactive agents should prevent the specific cardiovascular changes.

Prior work has demonstrated that criteria 1 and 2 are satisfied by bradykinin in spontaneous sepsis and experimental endotoxemia in primates. This vasoactive agent is generated in man and the monkey (9-13, 19), and its presence correlates with the cardiovascular changes occurring in endotoxemia (9, 10). At the present time there are no specific kinin-blocking drugs or agents which prevent kinin generation without also influencing other enzyme-substrate systems. In the absence of specificity, it is impossible to define the relative importance of inhibition of the kinin system in the effects of these agents. Until more specific inhibitors of the kinin system are developed it remains impossible to test criterion 4 adequately. In these experiments we have examined the effects of bradykinin on the circulation and have tested criterion 3.

Dose-response analysis revealed a linear relationship between dose of bradykinin infused and fall in arterial blood pressure. At the doses we infused, circulating levels of bradykinin were closely similar to those measured in venous blood during experimental endotoxemia in the primate (9). As previously noted, several groups of investigators have demonstrated the presence of bradykinin in the circulation in both man and the subhuman primate (9-12). Shah et al. (20) demonstrated very low levels of this peptide in the dog but speculated that the amount present was too small to be of importance. Others (21) have not found any kinin in endotoxemia in the dog. Their methods,
However, are 100-fold less sensitive than those presently in use in our laboratory and are inadequate to measure the levels of bradykinin spontaneously generated during endotoxemia. The dog clearly responds differently to acute endotoxemia than does the primate (22, 23). In addition, the kinin-generating systems of the dog have different specificities than those of the primate (24). The absence of high levels of kinin in this species does not, therefore, directly relate to the role of kinin in the shock syndrome in the primate.

An explanation for the recovery of resistance between 3 and 10 minutes of bradykinin infusion lies in the ability of kinins to release catecholamines directly from the adrenal medulla (25) or to activate the sympathetic nervous system through the baroreceptor reflex pathway (26). With autonomic blockade, resistance does not return to baseline after 10 minutes of bradykinin infusion (Table 2), confirming the importance of compensatory autonomic reflexes (26-28). Further evidence of activation of the sympathetic nervous system by effects of bradykinin is provided by evaluation of the pattern of regional distribution of blood flow which occurred during kinin infusion. Forsyth et al. (29, 30) have documented the pattern of regional blood flow and resistance associated with the activation of sympathetic nervous system by hemorrhage or hypothalamic stimulation in monkeys. Their results are consistent with results in other species and man (31-34). This sympathetic reflex-mediated pattern demonstrates preservation of blood flow to the heart, brain, liver and lung at the expense of less vital structures such as skin, bone, muscle, and abdominal viscera. The monkeys receiving only bradykinin developed a pattern of blood flow almost identical to that seen with sympathetic reflex activation, as in hemorrhage. The cardiovascular response to exogenous bradykinin infusion is complex. It is apparent that the systemic and regional patterns of blood flow and resistance found during kinin infusion are not the same as those seen in hemorrhage and autonomic blockade. The results of this study, together with the literature, extend the findings of Forsyth et al. (29, 30) and confirm the importance of kinins in the hyperdynamic response to hemorrhage and hypotension. The ability of kinins to activate both the sympa-thetic nervous system and the baroreceptor reflex pathway suggests that they play a role in the regulation of arterial pressure in response to hemorrhage or to hypotension.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Bradykinin</th>
<th>Ganglionic blockade</th>
<th>Bradykinin + ganglionic blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cardiac output (L/min)</td>
<td>Blood flow (ml/min 100 g tissue⁻¹)</td>
<td>Resistance (mm Hg./l. min⁻¹ 100 g tissue⁻¹)</td>
</tr>
<tr>
<td>Heart</td>
<td>146</td>
<td>196</td>
<td>86</td>
</tr>
<tr>
<td>Brain</td>
<td>116</td>
<td>79*</td>
<td>102</td>
</tr>
<tr>
<td>Kidney</td>
<td>138*</td>
<td>96</td>
<td>86*</td>
</tr>
<tr>
<td>Skin</td>
<td>60*</td>
<td>44*</td>
<td>208</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>64*</td>
<td>46*</td>
<td>197*</td>
</tr>
<tr>
<td>Stomach</td>
<td>92*</td>
<td>65*</td>
<td>134</td>
</tr>
<tr>
<td>Spleen</td>
<td>74*</td>
<td>33*</td>
<td>415*</td>
</tr>
<tr>
<td>Liver (hepatic artery)</td>
<td>180*</td>
<td>125*</td>
<td>71*</td>
</tr>
<tr>
<td>Bone</td>
<td>75*</td>
<td>55*</td>
<td>177</td>
</tr>
</tbody>
</table>

Values are given as percent of baseline values in ten monkeys.

*P < 0.05 (Mann-Whitney U-test) compared to changes in seven control monkeys.
endotoxemia. The sustained decrease in peripheral vascular resistance which has been demonstrated in the early shock state is not reproduced by kinin infusion, nor is the specific pattern of regional blood flow characteristic of endotoxemia (7). Bradykinin alone does not reproduce the hemodynamic consequences of endotoxemia, and thus cannot be the exclusive vasoactive mediator of the endotoxin shock state.

Another possibility is that endotoxin (or a released product) can, in some way, influence the autonomic nervous system and inhibit cardiovascular reflexes. One precedent for such an effect of endotoxin is its ability to release leukocyte pyrogen (35), which operates via a hypothalamic pathway to produce fever. Trank and Visscher (14) have demonstrated an influence of endotoxin on baroreceptor function that could contribute to an autonomic dysfunction during endotoxemia. Blattberg and Levy (36) have demonstrated increased vagal activity in dogs given endotoxin, and it is equally possible that a more complex effect of endotoxin may be responsible for the lack of response to low peripheral vascular resistance.

The data from the present experiments support the concept that the autonomic nervous system functions abnormally during endotoxemia. Autonomic blockade alone resulted in generalized vasodilation and decreased flow to most organs. The addition of bradykinin infusion during continued blockade increased resistance in the spleen, whereas resistance declined further in brain and kidney. Although these changes cannot be claimed to prove that the autonomic nervous system is functioning abnormally during endotoxemia, it is of interest that the only regional vascular bed exhibiting increased resistance during experimental endotoxemia is the spleen (7). These results suggest that vasodilators released by endotoxin and simultaneous distortion of autonomic reflex responsiveness could combine to produce the unique cardiovascular events of the endotoxin shock state.

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References


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