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Influence of Endotoxin on the Intrarenal Distribution of Gentamicin, Netilmicin, Tobramycin, Amikacin, and Cephalothin

MICHEL G. BERGERON* AND YVES BERGERON

Infectious Diseases Service, Le Centre Hospitalier de l'Université Laval, Québec, Québec, Canada

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Multiple factors may modify the pharmacokinetics of aminoglycosides and increase their nephrotoxic potential. In this study, we investigated the influence of *Escherichia coli* endotoxin on the renal handling of several aminoglycosides and one cephalosporin. Drug levels in the renal parenchyma, as well as several parameters of renal function and histology, were compared in rats treated with endotoxin (0.25 mg/kg) and normal rats treated with either gentamicin (10 mg/kg), netilmicin (10 mg/kg), tobramyin (10 mg/kg), amikacin (50 mg/kg), or cephalothin (100 mg/kg). Blood pressure and pulse rate were recorded. Endotoxin was associated with a decrease in the half-life and in the apparent volume of distribution of gentamicin. The endotoxin-injected animals accumulated significantly ($P < 0.05$) more aminoglycosides in their kidneys than the normal animals. The amount of cephalothin recovered in the renal parenchyma was identical in both groups. Slight decreases in the glomerular filtration rate and renal plasma flow were observed after endotoxin treatment. Blood pressure and cardiac frequency were minimally affected by endotoxin. No histological lesions were observed by light microscopy in animals receiving endotoxin. Thus, endotoxin modifies the renal handling of aminoglycosides in the absence of any major physiological disturbance or histological change. By increasing the total amount of drug within the kidneys, endotoxin might increase the nephrotoxic potential of aminoglycosides.

Previous studies done in our laboratory revealed that the intrarenal distribution of gentamicin was disturbed in experimental pyelonephritis due to *Escherichia coli* (5). A greater accumulation of drug was observed in the cortex and medulla of pyelonephritic animals than in the kidneys of noninfected animals. Pyelonephritic kidneys were also shown to be more susceptible to the nephrotoxic potential of gentamicin than normal kidneys (2). Pyelonephritic animals treated with gentamicin showed signs of renal damage which were more striking than those observed in normal treated rats. We considered the possibility that endotoxin liberated during antibiotic therapy (13, 25) might have disturbed the intrarenal pharmacokinetics of gentamicin and acted concomitantly with gentamicin on tubular cells to potentiate the toxicity of this antibiotic. The purpose of the present study was to evaluate the influence of endotoxin on the intrarenal distribution of aminoglycosides.

MATERIALS AND METHODS

Experimental model: distribution studies. Female Sprague-Dawley rats weighing 175 to 200 g were used for all experiments. Sixteen groups of 5 to 11 animals were anesthetized by a single intraperitoneal injection of sodium pentobarbital (45 mg/kg), followed 2 h later by a second dose of 20 mg/kg. Four animals, two controls and two endotoxin-treated rats, were used in parallel each day. Catheters were inserted into the right jugular vein for infusion of solutions. *E. coli* O127:B8 endotoxin (0.25 mg/kg; Difco Laboratories, Detroit, Mich.) or normal saline was infused intravenously over a period of 15 min at the beginning of the experiment. This low dosing of endotoxin was chosen to avoid major physiological disturbances in the Sprague-Dawley rats. In preliminary studies in which we used a dose of 0.5 mg/kg, we observed changes in blood pressure and pulse which were greater than the changes observed after doses of 0.25 or 0.05 mg/kg, which were associated with minor physiological changes. Thus, 0.25 mg/kg was chosen as the optimal dose for our experiments.

At 3 h after the initial infusion was given, an intravenous bolus of gentamicin (10 mg/kg; Schering Canada, Pointe-Claire, Quebec, Canada), netilmicin (10 mg/kg; Netromycin; Schering Canada), tobramycin (10 mg/kg; Nebcin; Eli Lilly Canada, Toronto, Ontario, Canada), amikacin (50 mg/kg; Amikin; Bristol-Myers, Candiac, Quebec, Canada), or cephalothin (100 mg/kg; Keflin; Eli Lilly Canada) was given. Normal saline was infused at a rate of 1.15 ml/h throughout the experiments.

Blood was removed by cardiac puncture and centrifuged 0.5, 1, 2, and 4 h after injection of gentamicin and 1 h after injection of the other drugs. Kidneys were removed at the same time, separated into cortex, medulla, and papilla, and homogenized in 0.1 M phosphate buffer (pH 7.4). Urine was also collected. The concentrations of drugs were determined by a standard disk biological assay (4). All assays were done in triplicate on tryptic soy agar (Difco) by using *Bacillus subtilis* as the test organism. Standard curves for the antibiotic assays were prepared with serum for serum, with physiological saline for urine, and with cortex, medulla, and papilla homogenates for the renal tissue. The level of recovery of the aminoglycosides after known amounts of drug-free homogenates were added was 98 ± 1.6% (3), while the level of recovery of cephalothin was 93 ± 6% (unpublished data).

Pharmacokinetics. The elimination rate constant (K_{el}), the elimination half-life ($t_{1/2}$; $t = \ln 2/K_{el}$), the area under the curve ($AUC_{0-\infty}$), and the volume of distribution (V_{area}; V_{area} = dose of gentamicin/[$AUC_{0-\infty} \cdot K_{el}$]) were determined by using model 1 (one compartment with bolus input and first-order output) of Pc Nonlin software (Statistical consult-

* Corresponding author.

TABLE 1. Serum concentrations of gentamicin in normal and endotoxin-injected rats

Time (h)	Serum concn (μg/ml) in:	
	Normal rats	Endotoxin-treated rats
0.5	9.8 (0.2)[a]	14.1 (2.9)
1.0	6.6 (0.9)	6.7 (1.3)
2.0	2.7 (0.5)	3.8 (0.7)
4.0	0.2 (0.2)	0.9 (0.4)

[a] The values in parentheses are standard errors of the mean.

ants, Lexington, Ky.). The total plasma clearance was calculated as follows: dose of gentamicin/$AUC_{0-\infty}$.

Physiological studies. Blood pressure and cardiac frequency were recorded with a model R711 physiograph (Beckman Instruments, Inc., Fullerton, Calif.) for animals receiving either endotoxin or normal saline from 0 to 7 h after endotoxin injection.

Renal function was also evaluated in normal and endotoxin-treated animals. Four rats of each group were anesthetized with pentobarbital (45 mg/kg). The left carotid artery and the right jugular vein were canulated with type PE-50 and PE-20 polyethylene tubing (Clay Adams), respectively. The former was used to sample blood, while the latter was used for infusion. At 3 h after an injection of endotoxin (0.25 mg/kg) or normal saline, a gentamicin (10 mg/kg) bolus was given. After gentamicin treatment a 1-ml bolus of 0.85% NaCl containing ^{14}C-labeled inulin (0.10 mCi/100 ml; New England Nuclear Corp., Boston, Mass.), ^{3}H-labeled *p*-aminohippuric acid (PAH; 0.50 mCi/100 ml; New England Nuclear Corp.), unlabeled inulin (1 mg/ml), and unlabeled PAH (10 mg/ml) as infused at a rate of 0.28 ml/min. This bolus was immediately followed by continuous infusion of the same solution at a rate of 0.052 ml/min; 1 h later, urine was collected for three sequential 30-min periods from the right and left ureters, which were separately canulated with type PE-10 tubing.

Blood samples were taken at the beginning and the end of each urine collection period. At the end of the last period, blood was also taken from the renal vein to measure the renal plasma flow. ^{14}C-labeled inulin and ^{3}H-labeled PAH were measured with a Beckman model Ls 7500 liquid scintillation counter by using a double-labeled program and automatic quench correction. The glomerular filtration rate was determined by inulin clearance. PAH clearance and PAH secretion were also evaluated. Renal plasma flow (RPF) was calculated by using the following equation: RPF = [(PAH in urine − PAH in renal venous plasma) × *V*]/(PAH in arterial plasma − PAH in renal venous plasma), where *V* was the urine flow (in milliliters per minute).

Histology. Histological observations were made by using kidneys from animals that received either no endotoxin or 0.25 mg of endotoxin per kg. The animals were sacrificed 3 h, 4 h, or 1 day after endotoxin treatment. At the time of sacrifice, a midline abdominal incision was made, and a type 21G butterfly needle was inserted into the aorta above the renal arteries. After perfusion of 10 ml of Krebs-Ringer solution (pH 7.4) at a rate of 7 ml/min, 100 ml of 2% glutaraldehyde (pH 7.4) was infused into the live animals. After the in vivo glutaraldehyde fixation, the kidneys were excised and placed in the same fixative for 2 h. Cubes (1 mm^3) were removed from the cortex and medulla and were left overnight in the same fixative at 4°C. After washing with 0.1 M phosphate buffer (pH 7.4), the cubes were further fixed in 2% osmium tetroxide for 3 h at 4°C, dehydrated in ascending grades of alcohol, and embedded in type 502 araldite resin. Thick sections (1 μm) were cut with a LKB Ultratome III, stained with toluidine blue, and examined by using a blind code to identify gross lesions.

Statistics. Data are presented as means ± standard errors of the mean. The statistical significance of differences between groups was determined by using the unpaired Student *t* test.

RESULTS

Aminoglycoside pharmacokinetics after endotoxin treatment. (i) Serum. The concentrations of gentamicin in sera are shown in Table 1. The peak levels were 9.8 ± 0.2 μg/ml in normal rats and 14.1 ± 2.9 μg/ml in endotoxin-treated animals 30 min after injection. The mean concentrations at each time point were higher after endotoxin treatment but did not differ significantly from control values. The serum levels of netilmicin, tobramycin, amikacin, and cephalothin after 1 h are shown in Table 2. Significant differences were observed with netilmicin ($P < 0.001$), tobramycin ($P < 0.01$), and amikacin ($P < 0.001$), while the cephalothin levels were not statistically different.

The pharmacokinetic data for the gentamicin-treated and endotoxin-plus-gentamicin-treated groups are shown in Table 3. No significant differences were observed for the area under the concentration-time curve, elimination half-life, and volume of distribution values after endotoxin treatment. The mean total plasma gentamicin clearance was slightly lower after endotoxin treatment.

(ii) Kidneys. Figure 1 shows the concentrations of gentamicin in the renal cortex, medulla, and papilla of normal and endotoxin-injected animals. The levels of drug in the cortex and medulla were always higher in the endotoxin-treated animals. Table 4 shows the area under the concentration-time curve values for gentamicin within the cortex, medulla, and papilla. There were significant differences

TABLE 2. Serum concentrations of netilmicin, tobramycin, amikacin, and cephalothin in normal and endotoxin-treated rats 1 h after injection

Antibiotic	Dose (mg/kg)	Serum concn (μg/ml) in:	
		Normal rats	Endotoxin-treated rats
Netilmicin	10	3.3 (0.2)[a]	7.0 (0.7)
Tobramycin	10	4.0 (0.6)	10.2 (1.5)
Amikacin	50	19.2 (1.4)	30.9 (1.4)
Cephalothin	100	4.1 (2.2)	9.2 (2.3)

[a] The values in parentheses are standard errors of the mean.

TABLE 3. Area under the serum curve, elimination half-life, volume of distribution, and total clearance of gentamicin in normal and endotoxin-injected animals

Group	$AUC_{0-\infty}$ (μg · h/ml)[a]	Elimination half-life (h)	Volume of distribution (ml/kg)	Total clearance (ml/min per kg)
Normal	17.8 (1.1)[b]	0.81 (0.10)	659 (57)	9.4
Endotoxin treated	21.0 (2.2)	0.60 (0.16)	411 (96)	8.4

[a] $AUC_{0-\infty}$, Area under the concentration-time curve.
[b] The values in parentheses are standard errors.

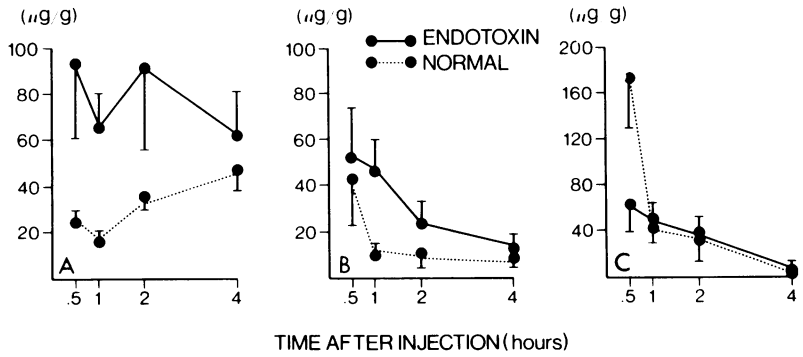

FIG. 1. Concentrations of gentamicin in the kidney cortex (A), medulla (B), and papillia (C) of normal and endotoxin-treated animals at 0.5 to 4 h after injection of 10 mg/kg.

between the endotoxin-treated and untreated animals (cortex, $P < 0.02$; medulla, $P < 0.01$).

Figure 2 shows the total amount of the four aminoglycosides studied and of cephalothin in the kidneys of rats at 1 h postinjection. All of the aminoglycosides (gentamicin, netilmicin, tobramycin, and amikacin) accumulated to a significantly greater degree in the endotoxin-treated group than in the controls ($P < 0.05$). Cephalothin was not significantly affected by endotoxin.

(iii) Urine. The urine concentration and total amount of gentamicin excreted are shown in Table 5. In the first hour, less antibiotic was recovered in the urine of the endotoxin-treated group than in the urine of the untreated group (24.8 ± 6.4 and 31.9 ± 3.6%, respectively); an even more striking difference was observed during the second hour of collection (5.9 ± 0.9 and 15.8 ± 1.5%, respectively; $P < 0.001$). During the 3 h of urine collection, the levels of recovery of gentamicin in the urine of normal and endotoxin-treated rats were 51.7 and 32.1%, respectively. Data for the other antibiotics are shown in Table 6.

The average volumes of urine produced during the first hour were 0.77 ± 0.30 ml in normal rats and 0.5 ± 0.04 ml in endotoxin-treated rats ($P < 0.01$); the average volumes produced during the second hour were 0.73 ± 0.13 and 0.32 ± 0.04 ml, respectively ($P < 0.01$).

Physiological studies. (i) Blood pressure and pulse rate. Following endotoxin treatment, systolic pressure was unchanged. The diastolic pressure decreased from 80 mm of Hg in the normal rats to 61 mm of Hg in the endotoxemic animals at 2 h but was normal at 3 h. The pulse rate was more rapid in the endotoxin-treated animals between 2 and 3 h after injection of endotoxin but came back to normal thereafter.

(ii) Renal function. Table 7 shows the data for inulin clearance, PAH clearance and secretion, and renal plasma flow. A decrease of 29% in the glomerular filtration rate was observed in the presence of endotoxin, as measured by inulin clearance. A slight decrease (9%) was observed in the tubular secretion of PAH. The PAH clearance, which represented the number of milliliters of serum cleared from the presence of PAH in 1 min by glomerular filtration and tubular secretion, was also diminished. The renal plasma flow was 2.25 ± of 0.53 ml/min in normal animals and 1.59 ± 0.62 ml/min in endotoxin-treated rats. However, none of these changes was statistically significant.

Histology. No histological lesions were observed when light microscopy was used to examine animals that received endotoxin.

TABLE 4. Area under the gentamicin curve for cortex, medulla, and papilla of normal and endotoxin-injected rats

Group	Area under the gentamicin concentration-time curve from 0.5 to 4 h (μg · h/g) for:		
	Cortex	Medulla	Papilla
Normal	115 (6)[a]	40 (6)	129 (34)
Endotoxin treated	271 (50)[b]	99 (12)[c]	109 (19)[d]

[a] The values in parentheses are standard errors.
[b] $P < 0.02$ compared with the value for normal rats.
[c] $P < 0.01$ compared with the value for normal rats.
[d] Not significant compared with the value for normal rats.

DISCUSSION

There are very limited data on the influence of endotoxin on the pharmacology of antibiotics. In this study, although endotoxin was given in low doses which did not affect the pulse rate or blood pressure of treated animals, endotoxin treatment resulted in decreases in the glomerular filtration rate, PAH clearance, and renal plasma flow, which most likely were responsible for the increase in serum levels and the decrease in urinary concentrations and rates of recovery of all antibiotics, including aminoglycosides and cephalo-

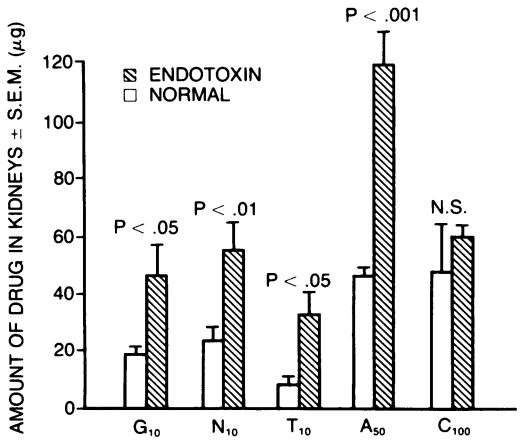

FIG. 2. Total amounts of drugs recovered in the whole kidneys of normal and endotoxin-injected rats 1 h following a single dose of gentamicin (10 mg/kg) (G_{10}), netilmicin (10 mg/kg) (N_{10}), tobramycin (10 mg/kg) (T_{10}), amikacin (50 mg/kg) (A_{50}), or cephalothin (100 mg/kg) (C_{100}). N.S., Not significant.

TABLE 5. Urinary concentrations of gentamicin and total amounts and percentages of the dose recovered in the urine of normal and endotoxin-injected rats

Time (h)	Urine concn (μg/ml)		Amt excreted in urine (μg)		% Of dose recovered	
	Normal rats	Endotoxin-treated rats	Normal rats	Endotoxin-treated rats	Normal rats	Endotoxin-treated rats
0–1	1,260 (333)[a]	997 (221)	600 (55)	516 (138)	31.9 (3.6)	24.8 (6.4)
1–2	490 (49)	368 (95)	332 (32)	104 (13)	15.8 (1.5)	5.9 (0.9)
3–4	144 (10)	94 (29)	78 (16)	43 (14)	4.0 (0.9)	2.4 (0.8)

[a] The values in parentheses are standard errors of the mean.

thin. Our data for gentamicin are identical to those of Wilson et al. (29), who noticed a decrease in the half-life and in the apparent volume of distribution of gentamicin in horses treated with *E. coli* O55:B5 endotoxin. A slight decrease in the extracellular fluid compartment volume could also explain the observations of these authors.

In contrast, Halkin et al. (14) observed a significant increase in the volume of distribution of gentamicin in endotoxin-induced pyrexia in rabbits. Halkin et al. believed that temperature-induced increases in the volume of distribution might be caused by altered rates of tissue perfusion or tissue binding of the drug. No fever response to endotoxin was observed in our experiments.

While the serum levels and the urinary excretion of all antibiotics, including cephalothin, were affected in a similar fashion by endotoxin, the intrarenal distribution of gentamicin, netilmicin, tobramycin, and amikacin was affected to a greater degree by endotoxin than the intrarenal distribution of cephalothin, suggesting that besides the physiological changes described above, endotoxin might have acted directly on the tubular transport of aminoglycosides without affecting the transport of the cephalosporin. In fact, the kidneys of endotoxin-injected rats accumulated more aminoglycosides than the kidneys of normal rats. These changes were more striking in the cortex than in the medulla.

Following filtration through glomeruli, gentamicin is believed to be reabsorbed in the proximal tubular cells by pinocytosis (26). The precise mechanism by which gentamicin attaches to the surface of tubular cells is not known, but both endotoxin and aminoglycosides seem to bind to receptor sites on phospholipids (1, 24). The phospholipid receptor seems to be an anionic binding site that is shared by polypeptides, amino acids, aminoglycosides, and cationic proteins that are reabsorbed (M. Sastrasinh, T. C. Knauss, J. M. Weinberg, and H. D. Humes, Kidney Int. **19**:213, 1981). Since endotoxin has been shown to increase the negative charge on the liposomes formed from different phospholipids (22), we cannot exclude the possibility that the binding between positively charged gentamicin (a polycationic drug) and the negatively charged phospholipids could have been enhanced by endotoxin. Thus, an increased incorporation of aminoglycosides within the kidney cells could explain the impressive renal uptake that we observed in the endotoxin-treated animals. In contrast, cephalothin, an organic acid which is handled by active tubular secretion, was not significantly affected by endotoxin.

Following their entrance within the tubular cells, aminoglycosides accumulate within the lysosomes (26), where they interfere with the normal catabolism of phospholipids, leading to cellular disturbance (1, 21). As with aminoglycoside therapy, lysosomes are also target organelles (6, 7, 16) of endotoxin. Endotoxin is distributed throughout the kidneys (9, 18), and autoradiographic studies have shown that lipid A, the active component of endotoxin, can be recovered within the tubular cells of the renal cortex (28). Manny et al. (17) observed that endotoxin induced rupture of lysosomal membranes and swelling of the mitochondria of proximal and distal tubules in the kidneys of dogs. Several of the subcellular changes observed in the endotoxin-treated animals are similar to the changes observed after aminoglycoside therapy (12, 19).

The interaction between drugs other than aminoglycosides and endotoxin has been described previously. Bradley and Bond (8) found that combinations of pactamycin and endotoxin administered simultaneously killed mice more rapidly than either drug alone; in vitro, the two drugs used in combination increased the in vitro fragility of renal lysosomes. Brown and Morrison (10) reported that simultaneous administration of actinomycin D to endotoxin-responsive mice increased by several orders of magnitude the susceptibility of these mice to the lethal effects of endotoxin. In contrast, Rifkind and Palmer (23) noted that three cationic cyclic polypeptide antibiotics, polymixin B sulfate, colistin sulfate, and tyrocidine hydrochloride, neutralized endotoxin lethality in chicken embryos.

As mentioned above, although the physiology parameters observed following endotoxin treatment were not significantly different from those of normal rats, we cannot exclude the possibility that the minor changes observed might have disturbed the uptake of aminoglycosides by the kidneys. The

TABLE 6. Urinary concentrations of netilmicin, tobramycin, amikacin, and cephalothin and total amounts and percentages of the dose recovered in the urine of normal and endotoxin-treated rats in the first hour after injection

Antibiotic	Dose (mg/kg)	Urine concn (μg/ml)		Amt excreted in urine (μg)		% Of dose recovered	
		Normal rats	Endotoxin-treated rats	Normal rats	Endotoxin-treated rats	Normal rats	Endotoxin-treated rats
Netilmicin	10	1,506 (97.1)[a]	463 (96.2)	735 (76)	307 (52)	35.5 (2.9)	14.8 (2.5)
Tobramycin	10	1,296 (86)	979 (278)	784 (28)	148 (54)	40.4 (1.4)	7.5 (2.8)
Amikacin	50	9,700 (854)	6,900 (739)	4,676 (388)	5,094 (906)	45.5 (3.5)	50.9 (9.6)
Cephalothin	100	10,200 (846)	2,950 (1031)	4,072 (91)	2,536 (749)	22.7 (0.6)	12.9 (3.8)

[a] The values in parentheses are standard errors of the mean.

TABLE 7. Effect of endotoxin on the glomerular filtration rate, PAH clearance, PAH transport, and renal plasma flow

Group	Glomerular filtration rate (ml/min)[a]	PAH clearance (ml/min)[a]	PAH transport (μmol/min)	Renal plasma flow (ml/min)
No endotoxin	0.360 (0.05)[b]	0.483 (0.07)	0.260 (0.11)	2.25 (0.53)
Endotoxin treated	0.254 (0.07)	0.395 (0.11)	0.236 (0.05)	1.59 (0.62)

[a] Values for 100 g of body weight.
[b] Mean values for the right kidneys. The values in parentheses are standard errors of the mean.

high serum antibiotic levels noticed in the endotoxin-treated animals could have resulted in better availability of all drugs for transport. If this were the case, endotoxin theoretically should have affected aminoglycosides and cephalosporins. Even though the mechanisms of transport of these drugs are so different, it is not unlikely that high serum levels could have favored the uptake of aminoglycosides by the kidneys without influencing the tubular uptake of cephalosporins. In contrast, the reduced glomerular filtration rate and renal plasma flow which resulted in higher serum levels should have reduced the amount of antibiotics delivered to tubular cells for transport. Although basolateral membrane transport of aminoglycosides is less important than apical membrane transport, endotoxin could have directly or indirectly allowed more drug to be taken up by the basolateral membrane of kidney cells.

Thomas (27) observed vasoconstriction-vasodilatation cycles after endotoxin treatment that were associated with modified renal plasma flow. After studying the effect of endotoxin on the renal function of dogs, Hinshaw et al. (15) concluded that the effect of endotoxin on kidneys is primarily vascular. Cavanagh et al. (11) observed a decrease in the renal artery flow of baboons after endotoxin treatment. Bradley (7) reported that the effects of endotoxin in kidneys appeared to be secondary to disseminated intravascular coagulation. McKay et al. (20) described fibrin deposits in the renal capillaries after endotoxin treatment. In contrast to the studies described above, neither ischemia nor any morphological modifications in the tubular cells or the vascular tree were observed following treatment with aminoglycosides or endotoxin or both.

In this study we found that low doses of endotoxin, which have been shown by Shenep and Mogan (25) to be liberated locally or systemically during gram-negative bacterial infections, especially when bacterial antibiotics are used, modify the renal handling of aminoglycosides in the absence of any major physiological disturbance or histological change. By increasing the total amount of gentamicin within the kidneys, endotoxin might increase the nephrotoxic potential of aminoglycosides.

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