Metabolism of the Nonhepatic Splanchnic Area in Baboons and the Effects of Endotoxin (36708)

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Most studies of endotoxic shock have used the dog as an experimental model. Of special concern has been a definition of the hemodynamic changes that set gram-negative endotoxic shock in motion. Based upon canine studies, a cardinal role has been assigned to the gut in the etiology of irreversible human septic shock. Experiments in dogs indicate that sepsis initiates the release of endogenous catecholamines which result in vasospasm in small arteries and veins in splanchnic visceral organs, especially in the intestine (1, 2). The resultant ischemia damages the intestine, permitting endotoxins to pass from lumen to blood. The initial hypotension thus becomes self-perpetuating.

Recent studies in species phylogenetically closer to man than the dog indicate that hemodynamic responses of dogs to endotoxin should be carefully scrutinized before being extrapolated to man (3–7). Of special note are the differences observed in mesenteric vascular responses to endotoxin in the rhesus monkey (8), the baboon (9), and in the dog. Dogs respond to endotoxin with abrupt but transient marked portal hypertension, early systemic arterial hypotension, and a profound decline in mesenteric blood flow. In the rhesus monkey and baboon, there is no massive sequestration of blood in the abdominal viscera, no striking portal hypertension and no decrease in mesenteric blood flow (8, 9). While mesenteric vascular resistance climbs progressively in canine endotoxemia (2), it falls progressively in the primate (8). In the dog, mesenteric vasoconstriction is a characteristic shock response; in the primate, the mesenteric vasculature dilates under the same conditions.

Tissue metabolism is dependent upon proper delivery of the various fuels of respiration to their sites of utilization. Thus, one would expect circulatory changes to bring about alterations in metabolic patterns as well. In view of the well-documented species differences between dogs and primates, it became of interest to study in the baboon the metabolic changes accompanying mesenteric vascular responses to endotoxin administration. It was our hope that by using a species phylogenetically close to man as our experimental model, we may gain better insight as to the underlying mechanism of human septic shock.

Methods. Experiments were performed on 8 adult male baboons (*Papio cynocephalus*), weighing between 23.0 and 32.5 kg, anesthetized with sodium pentobarbital (20 mg/kg). The animals were intubated with a cuffed endotracheal tube to permit expired gases to be trapped through outflow tubing containing Drierite or $CaSO_4$ to trap water vapor and $CaCO_3$.

Mean arterial blood pressure (MABP) was measured through a catheter introduced into the aorta via the femoral artery. The catheter was connected to a Statham pressure transducer, and pressure was registered on a Sanborn photographic recorder. All catheters were filled with saline and those placed in each femoral vein were used for separate infusions of the following solutions: (a) sodium pentobarbital diluted to 5 mg/ml, infused at 0.02 ml/min, and (b) free fatty acid mixture (FFA), consisting of an aqueous solution of 4% albumin carrying radio-labeled FFA (2 μCi/ml of 1-^{14}C-palmitic and 20 μCi/ml of 9-10-^{3}H-oleic acids) infused at the rate of 0.2 ml/min. A laporatomy was performed for placement of a catheter into the portal vein via a mesenteric branch.

A slow and continuous saline drip was givin into the portal vein catheter to keep it patent. No anticoagulant was administered to the animals. Infusions of FFA and Nembutal were begun as early as 235 min and as late as 90 min before endotoxin. Control blood samples were taken in all animals at approximately 10 min before endotoxin administration. Blood samples were grouped into those taken less than 60 min or greater than 60 min postendotoxin. The majority of the <60 min postendotoxin samples was taken 30 min after endotoxin administration. Most of the >60 min samples were drawn 90 min following endotoxin injection. Each blood sample was analyzed for concentrations of FFA, glucose, lactate, acetoacetate (AcAc) and β-hydroxybutyrate (β-OHB). FFA, glucose and lactate were determined as described previously (10). Measurement of AcAc and β-OHB was performed enzymatically by the methods of Williamson, Mellanby and Krebs (11). In addition, fluxes of palmitate and oleate were also evaluated.

At the end of the control period, an approximate LD_{90} of *E. coli* endotoxin, 4 to 5 mg/kg (Difco, Detroit) was intravenously administered. Animals were monitored over a range of 65 to 214 min postendotoxin.

Data presented in the tables had been calculated in the following manner:

(1) Extraction of ^{14}C-labeled FFA (E% 16:0, palmitic acid) =

$$\frac{[^{14}\text{C-FFA}]\ \text{A-P difference (dpm/ml)}}{[^{14}\text{C-FFA}]\ \text{art. (dpm/ml)}} \times 100.$$

E% 18:1 (oleic acid) was calculated in the same way as E% 16:0, except that this molecule was tagged with ^{3}H, therefore in the equation above [^{3}H-FFA] takes the place of [^{14}C-FFA].

(2) Removal of 16:0 fatty acid =

$$\frac{[^{14}\text{C-FFA}]\ \text{A-P difference (dpm/ml)}}{\text{sp act of arterial 16:0 FFA (dpm/}\mu\text{mole)}}.$$

(3) Oxidation of 16:0 fatty acid =

$$\frac{[^{14}\text{CO}_2]\ \text{P-A difference (dpm/ml)}}{\text{sp act of arterial 16:0 FFA (dpm/}\mu\text{mole)}}.$$

(4) Percentage of CO_2 production derived from FFA oxidation. It is assumed that all long chain fatty acids are oxidized in the same fashion as palmitic acid. The equation then becomes =

$$\frac{\text{FFA oxidation (}\mu\text{mole/ml)} \times 17}{[\text{CO}_2]\ \text{P-A difference (}\mu\text{moles/ml)}} \times 100;$$

where FFA oxidation is obtained as =

$$\frac{[^{14}\text{CO}_2]\ \text{P-A difference (dpm/ml)}}{\text{sp act of total arterial FFA (dpm/}\mu\text{mole)}}.$$

(5) FFA flux (μmoles/min) =

$$\frac{\text{Infused 1-}^{14}\text{C-palmitic or 9-10-}^{3}\text{H-oleic acid (dpm/min)}}{\text{sp act of arterial FFA (dpm/}\mu\text{mole)}}.$$

Results and Discussion. In order to assess metabolic changes due to endotoxin in the nonhepatic splanchnic area (NHSA), some values had to be established under normal, control conditions. Several indices of FFA metabolism and glucose uptake in NHSA of normal baboons are presented in Table I. Both FFA and glucose are taken up consistently by tissues in this region. The extent of extraction of palmitic (16:0) and oleic (18:1) acids is about the same, 22.6 and 18.7%, respectively. These values are in good agreement with those obtained in canine intestinal segments, namely 22.2 and 21.7% (18).

The absolute amount of palmitic acid removed is 20.3 nmoles/ml, of which 10%, or 2.0 nmoles/ml is oxidized. Intestinal segments

TABLE I. FFA Metabolism and Glucose Uptake in NHSA of Normal Baboons.

A-P FFA[a]	35.3 ± 14.6[b]	(7)
E% palmitic acid	22.6 ± 3.9	(7)
E% oleic acid	18.7 ± 3.9	(8)
Palmitic acid removal	20.3 ± 3.9	(7)
Palmitic acid oxidation	2.0 ± 0.7	(6)
P-A CO_2	1550 ± 554	(8)
CO_2 (%) production derived from FFA oxidation	8.0 ± 2.8	(8)
A-P glucose	629 ± 207	(7)

[a] All values other than percentage are expressed in nmoles/ml. Numbers in parentheses refer to the number of animals.

[b] Mean ± SEM.

TABLE II. Various Hemodynamic Parameters Before and After Endotoxin Administration.[a]

	Before	After endotoxin	
		<60 min	>60 min
MABP	110 ± 3.3[b] (8)	47 ± 2.0 (6)	50 ± 7.7 (6)
HR	186 ± 12 (7)	196 ± 15 (3)	198 ± 18 (5)
HCT	44.3 ± 1.8 (8)	37.0 ± 3.5 (5)	38.4 ± 1.4 (6)

[a] MABP = mean arterial blood pressure; HR = heart rate; HCT = arterial hematocrit.

[b] Mean ± SEM. Numbers in parentheses refer to the number of animals.

in the dog oxidize about 24% of the FFA taken up (18).

In the baboon, 8% of the total CO_2 production by the NHSA can be accounted for by FFA oxidation. In dogs, 19.8% of total CO_2 production by the GI tract is derived from FFA (19), 22.1% by the mesenteric tissues (18).

The effect of endotoxin on some hemodynamic parameters is shown in Table II. Mean arterial blood pressure (MABP) and hematocrit (HCT) have decreased, heart rate (HR) did not change significantly following endotoxin administration in these anesthetized baboons.

Arterial concentrations of the various metabolites monitored—namely FFA, glucose, lactate, AcAc, βOHB—are shown in Table III. In addition, fluxes of palmitate (16:0) and oleate (18:1) are also presented. The arterial concentration of FFA decreased significantly at >60 min after endotoxin administration, the mean change being —0.116 ± 0.050 μmole/ml. In the dog, administration of endotoxin causes no significant changes in arterial FFA levels (12), but during hemorrhagic shock the mean arterial FFA concentration does decrease (13). The flux of both palmitic and oleic acids decreased substantially >60 min after endotoxin administration. This finding is similar to our earlier reports on the decrease of palmitic and oleic acid fluxes due to hemorrhage in dogs (13, 18).

Arterial glucose concentration remained unchanged at <60 min, and decreased at >60 min following endotoxin administration. The dog's glucose level follows a similar pattern in endotoxic shock (12). The hypoglycemia observed during terminal phases of shock is attributed to depletion of liver glycogen and failure of gluconeogenesis to keep pace with progressively increasing carbohydrate utilization. It cannot be ascribed to high circulating serum insulin levels, since it has been shown that during septic shock in baboons, induced by infusion of live *E. coli* organisms, insulin levels dropped immediately and remained low throughout the 4 hr of study (14). Similarly, the serum insulin level is not elevated in baboons in hemorrhagic shock either (15). The mean decrease of arterial glucose observed by us in this study >60 min after endotoxin administration is at variance with published results of Cryer, Herman and Sade (14), who noted hyperglycemia starting 15 min after injection of live *E. coli* organisms into baboons. Our experiments were carried out under pentobarbital anesthesia, whereas in the Cryer, Herman and Sade study the baboons were tranquilized. In the present study, the shock-inducing agent was *E. coli* endotoxin, while Cryer, Herman and Sade used live *E. coli* organisms. Thus the divergent response in blood sugar level may be attributed to one or both of these differences.

The arterial lactate concentration increased significantly both at <60 min and >60 min after endotoxin administration in these baboons, just as it did in the canine model (12).

In addition to peripheral organs, the liver appears to contribute significantly to development of hyperlactemia in low flow states either by failing to clear lactate from the blood when hepatic O_2 consumption is maintained or even by actual lactate production when O_2 consumption is low (16). Even though the mesenteric blood flow and portal

TABLE III. Arterial Concentrations of Various Metabolites and FFA Flux Before and After Endotoxin Administration.

	Control	After endotoxin			
		<60 min	Δ (exp.—contr.)	>60 min	Δ (exp.—contr.)
FFA	0.419 ± 0.099[a] (7)	0.412 ± 0.109 (6)	—0.057 ± 0.049 (5)	0.310 ± 0.082 (7)	—0.116 ± 0.050 (7)
FFA flux[b]	123 ± 35 (6)	88 ± 30 (4)	—8.8 ± 21.8 (4)	85 ± 36 (6)	—40.7 ± 15.5 (6)
FFA flux[c]	86 ± 27 (6)	43 ± 12 (4)	—32.3 ± 15.1 (4)	48 ± 24 (6)	—37.3 ± 13.3 (6)
Glucose	7.80 ± 0.56 (8)	8.07 ± 0.73 (7)	0.086 ± 0.70 (7)	5.71 ± 0.74 (7)	—1.70 ± 0.89 (7)
Lactate	1.79 ± 0.30 (8)	3.63 ± 0.68 (7)	1.60 ± 0.44 (6)	3.99 ± 0.64 (6)	2.43 ± 0.45 (6)
βOHB	0.060 ± 0.039 (8)	0.098 ± 0.066 (6)	0.017 ± 0.018 (6)	0.083 ± 0.048 (6)	0.0025 ± 0.19 (6)
AcAc	0.101 ± 0.025 (8)	0.093 ± 0.034 (7)	—0.015 ± 0.007 (7)	0.085 ± 0.024 (7)	—0.023 ± 0.016 (7)
AcAc/βOHB	1.80 ± 0.76 (4)	1.56 ± 0.90 (3)	—0.41 ± 0.16 (3)	1.32 ± 0.58 (4)	—0.48 ± 0.18 (4)

[a] Mean ± SEM. All concentrations are expressed in μmoles/ml. FFA and glucose are measured in plasma, the other metabolites in blood. Numbers in parentheses refer to the number of animals.

[b] Based on 1-^{14}C-palmitic acid.

[c] Based on 9-10-^{3}H-oleic acid.

pressure do not change in baboons during endotoxemia (9), it is conceivable that at the same time the metabolic commitments of the area are still compromised due to the release of one or more blood borne agents that may not exhibit vasoactive properties.

Significant decreases were observed in arterial AcAc concentration at <60 min, and in the AcAc/βOHB ratio both at <60 min and >60 min after endotoxin injection (Table III).

Changes in the AcAc/β-OHB ratio in the blood are difficult to interpret in the absence of tracer experiments. Since AcAc is the molecular species directly metabolized (17), the lower AcAc/β-OHB ratio observed after endotoxin injury suggests the possibility of increased utilization of ketone bodies by extrahepatic tissues.

The effects of endotoxin on the metabolism of the NHSA are shown in Table IV. Alterations in the NHSA metabolism of these baboons are not consistent and are elusive. However, endotoxin administration brings about metabolic adjustments in the whole animal, that are reflected by quite consistent changes in arterial concentrations of various metabolites (Table III).

It is worthy of note that even though the canine and nonhuman primate models provide some interesting contrasts in their mesenteric vascular responses to endotoxin administration, they share some of the systemic metabolic hallmarks of endotoxic shock, *e.g.*, lactacidemia and hypoglycemia. Hemodynamic deterioration of the NHSA is absent in baboons in endotoxic shock and so are predictable shifts in the pattern of metabolite flow and utilization.

Summary. Values of several parameters of lipid and carbohydrate metabolism were established in the NHSA of anesthetized baboons under normal conditions. The tissues in this area consistently remove FFA and glucose. About 20% of palmitic and oleic acids are extracted. Of the amount of palmitic acid taken up, 10% is oxidized. Eight percent of the total CO_2 produced by the NHSA can be accounted for by FFA oxidation.

The metabolic effects of *E. coli* endotoxin administration were studied in terms of alter-

TABLE IV. Changes in FFA Metabolism and Glucose Uptake in NHSA of Baboons Following Endotoxin Administration.

	After endotoxin	
	Δ < 60 min	Δ > 60 min
A-P FFA[a]	—14.0 ± 21.0[b] (4)	—31.0 ± 27.0 (5)
E% 16:0	—13.9 ± 4.7 (5)	10.7 ± 11.5 (5)
E% 18:1	—15.6 ± 8.6 (5)	4.0 ± 3.4 (5)
16:0 removal	8.8 ± 5.0 (5)	—12.1 ± 12.5 (5)
16:0 oxidation	—0.08 ± 0.4 (4)	—1.4 ± 1.5 (4)
P-A CO_2	360 ±400 (6)	810 ±710 (6)
CO_2 (%) production derived from FFA oxidation	—4.9 ± 3.5 (4)	6.4 ± 3.6 (5)
A-P glucose	10 ±264 (5)	—348 ±249 (5)

[a] All values other than percentages are expressed in nmoles/ml. Numbers in parentheses refer to the number of animals.

[b] Mean ± SEM.

ations in metabolite concentrations systemically and in the NHSA. Arterial FFA levels, as well as the flux of palmitic and oleic acids decreased substantially >60 min after endotoxin administration. Arterial glucose concentrations decreased at >60 min, lactate concentrations increased significantly, AcAc/β-OHB ratios decreased significantly, both at <60 min and >60 min following toxin injection. Metabolic changes in the NHSA were inconsistent.

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