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Fever Is Not Responsible for the Elevated Glucose Kinetics in Sepsis (42569)

CHARLES H. LANG,[1] GREGORY J. BAGBY, HOWARD L. BLAKESLEY, AND JOHN J. SPITZER

Department of Physiology, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, Louisiana 70112

Abstract. Previous studies have suggested that alterations in the classical neuroendocrine system may not be responsible for the increased glucose metabolism observed during hypermetabolic sepsis. The purpose of the present study was to determine whether inhibition of the cyclooxygenase pathway with indomethacin, which prevents the production of arachidonic acid metabolites by this pathway and the sepsis-induced increase in body temperature, would abolish the increases in glucose appearance (Ra), recycling, and hyperlactacidemia. Sepsis was induced in chronically catheterized conscious rats by multiple injections of live *Escherichia coli* via a subcutaneous catheter. Septic animals received iv injections of indomethacin (5 mg/kg) every 6–8 hr to block the cyclooxygenase pathway. Glucose kinetics were assessed in 24-hr fasted rats using a constant iv infusion of [6-^{3}H]- and [U-^{14}C]glucose. Treatment with indomethacin prevented the 1–2°C increase in body temperature observed in septic animals. Septic rats exhibited an elevated plasma lactate concentration and increased rates of glucose appearance and recycling. The sepsis-induced alterations in these variables were not attenuated by indomethacin. These results suggest that neither elevated body temperature nor the generation of arachidonic acid metabolites of the cyclooxygenase pathway is responsible for increasing glucose production in hypermetabolic septic rats.

Alterations in carbohydrate metabolism and fever are hallmarks of gram-negative septicemia (1, 2). Clinical and experimental studies have demonstrated increased rates of glucose production, recycling, and metabolic clearance, as well as hyperlactacidemia, during the hypermetabolic phase of sepsis (3–5). The mechanism for this increased glucose metabolism and its relationship to the febrile response, however, have not been elucidated.

In the past several years evidence has been presented supporting the view that prostaglandins may be responsible for many of the manifestations seen during sepsis and endotoxemia (6). It is well documented that the blood concentrations of various products of the cyclooxygenase pathway are elevated during these conditions (6–8). In addition to their cardiovascular effects the prostanoids also produce a wide range of metabolic alterations when administered to control animals. It appears that prostaglandins of the E series have the most pronounced affect on carbohydrate metabolism and that many of these alterations are similar to those observed during sepsis. The intravenous infusion of PGE has been shown to increase the rate of gluconeogenesis, decrease hepatic glycogen synthase activity, increase phosphorylase activity, and alter insulin and glucagon secretion (9–11).

In addition to the direct effects of prostanoids, prostaglandin E_2 (PGE_2) may alter carbohydrate metabolism secondarily through increasing body temperature. The febrile response, which is a characteristic manifestation of hypermetabolic sepsis, also appears to be mediated by PGE_2. Considerable evidence suggests that circulating interleukin-1 (IL-1), produced by bacterially stimulated phagocytic mononuclear cells, acts within the brain to increase PGE_2 production which alters the thermoregulatory set point (12). Since the sepsis-induced increase in body temperature has been shown to be related to a generalized elevation in whole-body metabolism, it is possible that fever alone is responsible for or is a major contributor to the elevated glucose metabolism seen in sepsis. Therefore, the purpose of the present study was to determine if fever was responsible for increased glucose metabolism observed during the hypermetabolic phase of gram-negative sepsis. This was investigated by

[1] To whom all correspondence and reprint requests should be addressed.

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determining if blockade of the cyclooxygenase pathway with indomethacin, and the concomitant reduction in fever, abolished the increased glucose metabolism observed in sepsis.

Materials and Methods. *Animal preparation.* Male Sprague–Dawley rats (250–325 g, Charles River) were housed in a controlled environment, exposed to a 12/12-hr light/dark cycle, and provided with standard rodent chow (Purina No. 5001) and water for at least 1 week before initiation of the experimental procedures. Animals were anesthetized with ether and catheters were implanted in the left carotid artery and the right jugular vein. An additional catheter was implanted subcutaneously on the dorsal surface of the animal for the subsequent administration of live *Escherichia coli.* All surgery was performed using aseptic techniques and control animals have been shown to remain nonseptic for up to 5 days postoperatively (13). Following catheterization, animals were returned to individual cages and provided water *ad libitum.*

Induction of sepsis. Sepsis was induced by multiple subcutaneous injections of live *E. coli* (026:B6, American Type Culture Collection). Fresh bacterial suspensions were prepared daily using standard procedures. An aliquot of the final suspension was serially diluted and each dilution was plated for quantitation. The remainder of the suspension was used for induction of sepsis.

Live *E. coli* (2×10^{10} organisms) were injected subcutaneously via the implanted catheter at noon and 5 PM on the day of surgery and at 8 and 11 AM on the following day. This regime produced a hypermetabolic septic state, as evidenced by the sustained elevation of body temperature, but resulted in no lethality during the experimental period. Time-matched control animals received an equal volume (1 ml) of sterile saline injected subcutaneously. Experiments were started after the final injection of *E. coli.* All animals were conscious and unrestrained throughout the study of glucose kinetics.

Experimental protocol. Four groups of animals were included in this study: nonseptic rats injected with indomethacin, nonseptic rats injected with vehicle, septic rats injected with indomethacin, and septic rats injected with vehicle. Indomethacin (5 mg/kg body wt, dissolved in 0.1 *M* sodium carbonate) was injected intravenously 1 hr prior to the first injection of *E. coli* and then every 6–8 hr thereafter (i.e., 5 and 11 PM on the day of surgery, and 7 AM on Day 2). The dose and frequency of administration of indomethacin were based on its ability to reduce the sepsis-induced hyperthermia and its inhibition of the hypotensive response produced by exogenously administered arachidonic acid (see Results). This dose of indomethacin has also been shown to prevent the sepsis- and endotoxin-induced elevation in plasma prostaglandins, thromboxane, and prostacyclin (8, 14). Preliminary studies indicated that injection of this volume and concentration of sodium carbonate did not produce detectable alterations in blood pH in either septic (7.51 ± 0.01) or nonseptic (7.51 ± 0.01) animals, compared to time-matched saline-injected control rats (7.50 ± 0.02).

Within 1 hr after the last injection of *E. coli* (or saline) a continuous iv infusion of tracer quantities of radiolabeled glucose was begun to assess glucose kinetics. The infusate was delivered at a constant rate of 0.7 ml/hr and contained [6-^{3}H] glucose (25 μCi/ml) and [U-^{14}C]glucose (5 μCi/ml). Preliminary studies showed that isotope equilibrium was achieved within 90 min; therefore, arterial blood samples (0.3 ml) were collected at 120 and 140 min. Blood samples were used to determine plasma glucose, lactate and glucose specific activity. Mean arterial blood pressure was monitored by a transducer attached to the arterial catheter. Colonic temperature was determined, using a flexible thermistor (Edwards Laboratories), prior to injections of *E. coli* both on Day 2 and at the end of the experimental protocol.

Arterial blood was deproteinized and neutralized, and the supernatant was analyzed enzymatically for glucose and lactate (15). Plasma glucose specific activity was determined by separating anionic radioactive products from glucose with ion-exchange resin (Amberlite MB-3) followed by evaporation to remove ^3H_2O (4) prior to liquid scintillation counting (LSC 7500, Beckman).

The rate of glucose appearance (Ra; μmole/min/kg) into the plasma was calculated from the non-steady-state equations of Steele (16), using the infusion rate for the ^{3}H-labeled tracer (dpm/min/kg) and the plasma [^{3}H]glucose specific activity (dpm/μmol). The rate of glu-

cose carbon recycling was calculated as the difference between the rates of appearance using [^{3}H]glucose and the [^{14}C]glucose, divided by the [^{3}H]glucose Ra. The former label was chosen because the ^{3}H in the six position is not recycled (17). In contrast, the [^{14}C]glucose gives rise to labeled gluconeogenic precursors, such as lactate, pyruvate, and alanine, which can be reincorporated into glucose by the liver.

Data were analyzed using one way analysis of variance followed by Newman–Keuls' test to determine treatment effect. Statistical significance was set at $P < 0.05$.

Results. *Efficacy and duration of drug blockade.* A bioassay, involving arachidonic acid-induced hypotension, was utilized to determine an appropriate dose and the frequency of administration for indomethacin. Animals with indwelling vascular catheters were injected iv with increasing doses of sodium arachidonate (0.5, 1, 2, and 3 mg/rat; Sigma) dissolved in 0.1 *M* sodium carbonate. The maximal reduction in mean arterial pressure was similar in nonseptic and septic rats (−3 ± 4, +3 ± 1; −26 ± 8, −13 ± 3; −45 ± 8, −43 ± 3; −40 ± 6, −35 ± 3 mm Hg, respectively) and dose-related. The time of onset and the duration of the hypotension at each dose were also not different between groups (data not shown). Table I indicates that the maximal hypotensive response was similar in magnitude after each of five successive injections of 2 mg of arachidonic acid, indicating no decrease in responsiveness to repeated injections of arachidonate. To test the duration of effective cyclooxygenase blockade, rats were injected iv with 2 mg of arachidonate at selected times after administration of indomethacin (5 mg/kg). The results indicate that indomethacin produced a complete blockade of the arachidonate-induced hypotensive response for at least 8 hr (Table I). Thereafter, the inhibition was only partially effective at preventing the decrease in blood pressure, and was lost altogether by 24 hr. Therefore, multiple doses of indomethacin were administered in order to provide a sustained blockade of cyclooxygenase pathway.

Blockade with indomethacin. Sepsis produced a 1–1.7°C increase in body temperature. Figure 1 illustrates that the dosage regime for indomethacin prevented this increase in body temperature. Indomethacin treatment did not alter temperature in nonseptic control animals. All animals appeared hemodynamically stable with mean arterial blood pressure (MABP) being similar in nonseptic and septic animals that received vehicle (107 ± 4 and 108 ± 6 mm Hg, respectively) and indomethacin (103 ± 4 and 120 ± 5 mm Hg, respectively). Heart rate was also not different among the four groups (375 ± 23 and 385 ± 19 beats/min for the vehicle-treated nonseptic and septic rats; 377 ± 18 and 402 ± 14 beats/min for the indomethacin-treated animals).

There was no significant differences in the

TABLE I. INHIBITION OF ARACHIDONIC ACID-INDUCED HYPOTENSION BY INDOMETHACIN

Group	Time after treatment with indomethacin or vehicle (hr)				
	2	5	8	12	24
Vehicle-treated nonseptic	−40 ± 5	−38 ± 4	−42 ± 3	−45 ± 5	−43 ± 4
Indomethacin-treated nonseptic	0 ± 5*	+2 ± 3*	−3 ± 4*	−22 ± 7*	−45 ± 8
Indomethacin-treated septic	−2 ± 4*	0 ± 3*	+3 ± 5*	−25 ± 5*	−37 ± 5

Note. Values are means ± SEM. N = 4–5 per group. Negative values represent maximal decreases in mean arterial blood pressure (MABP); positive values represent maximal increases in MABP. Absolute MABP is 112 ± 5, 103 ± 4, and 108 ± 6 mm Hg for the saline-treated nonseptic, indomethacin-treated nonseptic, and indomethacin-treated septic animals, respectively. Indomethacin was injected iv at a dose of 5 mg/kg. Vehicle-treated animals received an equal volume of 0.1 *M* sodium carbonate. Arachidonate was injected iv at a dose of 2 mg/rat. *$P < 0.05$ compared to time-matched vehicle-treated nonseptic animals. There were no statistical differences between indomethacin-treated septic and nonseptic rats at any time point. Values for indomethacin-treated animals were not different from zero at 2, 5, and 8 hr.

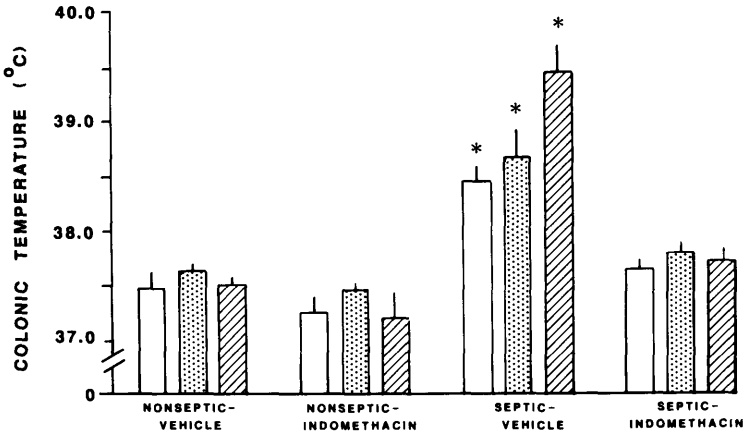

FIG. 1. Colonic temperature in nonseptic and septic rats treated with vehicle or indomethacin. Temperatures were taken at 7 AM (□), 11 AM (□), and 2 PM (▨) on the day glucose kinetic experiments were performed. These times represent 19, 24, and 27 hr after the first injection of *E. coli*. Temperatures at 7 and 11 AM were obtained prior to injection of *E. coli*. Values are means ± SEM; $N = 16$ and 6 for nonseptic and septic groups, respectively. $^*P < 0.05$ compared to nonseptic-vehicle values.

blood glucose concentration between nonseptic and septic vehicle-treated animals, and indomethacin did not significantly alter glucose concentration in either nonseptic or septic rats (Fig. 2). Plasma lactate concentrations were increased 50% in septic animals treated with sodium carbonate, compared to vehicle-treated nonseptic rats (Fig. 2). Indomethacin failed to have a significant effect on the sepsis-induced hyperlactacidemia.

Figure 3 illustrates the glucose kinetic data in nonseptic and septic rats with and without indomethacin treatment. The rate of glucose Ra was elevated in both the vehicle- and indomethacin-treated septic animals, compared to nonseptic-vehicle values. The rate of glucose recycling averaged 5.3 μmol/min/kg for both groups of nonseptic animals and was elevated in both the vehicle- and indomethacin-treated septic rats. Again, the cyclooxygenase inhibitor was not effective in blocking this change. The metabolic clearance rate (MCR) for glucose was elevated (62%) only in the septic animals receiving indomethacin. Vehicle-treated septic rats had clearance rates which were not different from nonseptic values. The reason for the apparent increase in glucose MCR in the indomethacin-treated septic rats is not clear. Since MCR is a derived value, it is possible that the small statistically nonsignificant alterations in the two measured parameters (e.g., a decreased glucose Ra and increased glucose concentration) resulted in a statistically significant fall in the MCR, which may be the

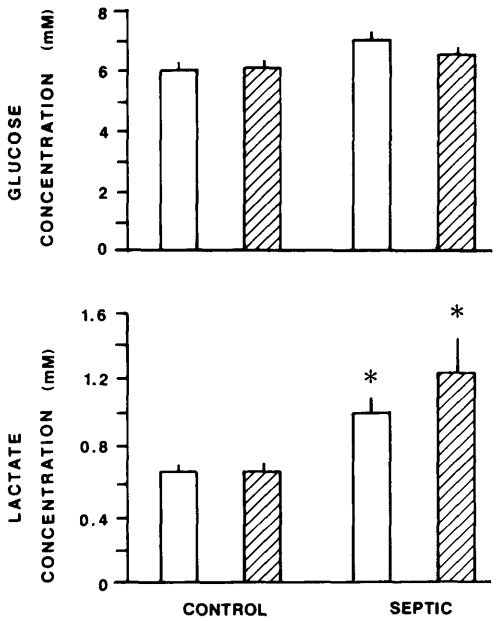

FIG. 2. Plasma glucose and lactate concentrations in nonseptic and septic animals administered vehicle (□) or indomethacin (▨). Values are means ± SEM; $N = 16$ and 6 for nonseptic and septic groups, respectively. $^*P < 0.05$ compared to nonseptic-vehicle values.

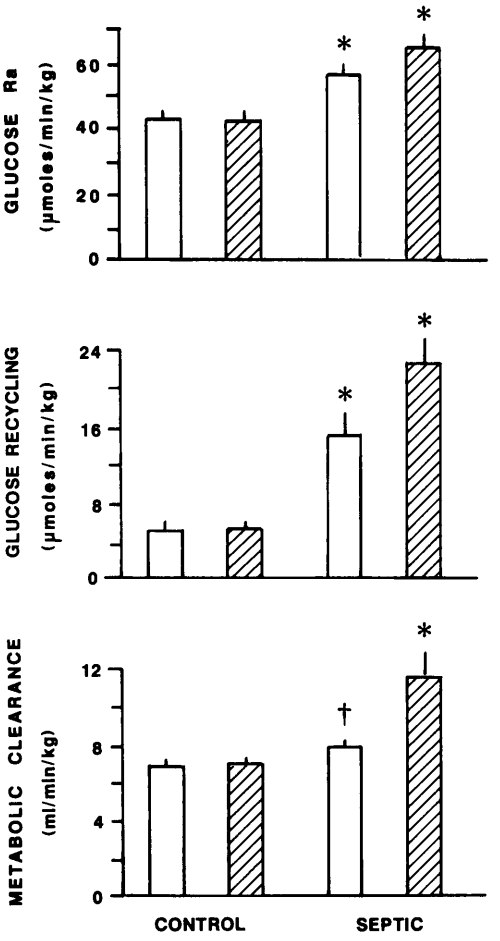

FIG. 3. Glucose kinetic data obtained during the infusion of [6-^{3}H]- and [U-^{14}C]glucose into nonseptic and septic rats administered vehicle (□) or indomethacin (▨). N = 16 and 6 for nonseptic and septic groups, respectively. *P < 0.05 compared to nonseptic-vehicle values. †P < 0.05 compared to septic-indomethacin values.

result of intergroup variability. In this regard, it should be noted that the absolute value for MCR (and recycling) in the indomethacin-treated septic rats is quite similar to that reported by us previously for untreated septic rats (4). The results of the present study, however, definitively indicate that the use of indomethacin to block the cyclooxygenase pathway failed to reverse the sepsis-induced increases in glucose Ra and recycling.

Discussion. The results of the present study indicate that the sepsis-induced increase in glucose metabolism was not associated with the elevated body temperature. Furthermore, the enhanced production of cyclooxygenase metabolites during sepsis also does not appear to be a prerequisite for the elevated rates of glucose appearance and recycling and the hyperlactacidemia. Although plasma concentrations of the various prostanoids were not determined, similar doses of indomethacin have been shown to inhibit increases in prostaglandins, thromboxane, or prostacyclin induced by sepsis (8), endotoxin (14), or carrageenin inflammation (18). The presence of the cyclooxygenase blockade was, however, verified in the present study by the absence of a hypotensive response to the intravenous injection of arachidonic acid.

We have previously demonstrated that the elevations in glucose metabolism seen using this relatively mild form of sepsis were not associated with the classical neuroendocrine system (19), since no changes in insulin, glucagon, or corticosterone were seen in these animals. Although plasma catecholamine levels were elevated by 50%, a complete α- and β-adrenergic blockade failed to prevent or reverse the sepsis-induced increases in glucose metabolism (19). Thus, it appears likely that other mechanisms must be responsible for these alterations. Evidence implicating macrophage-produced mediators in many of the pathophysiological consequences of sepsis and endotoxemia is mounting (20). While IL-1 or endogenous pyrogen has been shown to alter the plasma glucose concentration, as well as the insulin and glucagon levels (21), its effect on glucose kinetics has not been elucidated. However, other mediators, such as tumor necrosis factor, which is also released by monocytic cells in response to infection, have been shown to produce alterations in lipid and carbohydrate metabolism that are similar to those seen during sepsis (22).

Cyclooxygenase products of arachidonic acid could conceivably be mediators of the metabolic alterations induced by sepsis (6). Indications of their involvement in the sequela of these conditions fall into two categories. First, an increased plasma concentration of these substances, or their stable metabolites, is observed during sepsis and endotoxemia (6–8). Second, when PGE_1 or PGE_2 is administered systemically to man or animals, the alterations in carbohydrate metabolism mimic

those observed during hypermetabolic sepsis (1, 2). These PGE-induced alterations include an increased rate of glucose appearance, increased hepatic glycogen phosphorylase activity, decreased glycogen synthase activity, and insulin resistance (10–12). However, the present study indicates that treatment with indomethacin fails to alter *in vivo* glucose kinetics in the hypermetabolic septic rat and strongly suggests, but does not prove, that metabolites of the cyclooxygenase pathway may be relatively unimportant under these conditions in producing the metabolic consequences. These results are consistent with our previous *in vitro* findings, which also did not demonstrate any affects of indomethacin on glucose uptake or lactate release by muscles removed from endotoxemic rats (23). Inhibition of the cyclooxygenase pathway has also failed to reverse the acute-phase and protein catabolic responses induced by sepsis (24, 25) or burn (26).

Considerable evidence implicates PGE_2, a cyclooxygenase product of arachidonic acid, in the genesis of fever (12). The production of PGE_2 within the brain appears to be enhanced in response to circulating interleukin-1. By inhibiting the cyclooxygenase pathway and the subsequent production of PGE_2 indomethacin, as well as other nonsteroidal anti-inflammatory drugs (NSAIDs), can prevent or reverse the febrile response. The etiology of the elevated body temperature does not appear to be particularly important, since indomethacin has been shown to be antipyretic following the administration of endotoxin (27), muramyl dipeptide (28), or purified IL-1 (27). However, while NSAIDs reduce the elevated body temperature, they do not inhibit the production of IL-1 or its other biological activities (25, 27). In this regard, administration of ibuprofen, a specific cyclooxygenase inhibitor, can block the febrile response to IL-1, sepsis, or endotoxin, but failed to attenuate the acute-phase protein and trace metal responses to these challenges. Thus, the present study demonstrates that the elevated whole-body glucose kinetics observed during hypermetabolic sepsis can be dissociated from the elevated body temperature as well as can increases in cyclooxygenase products of arachidonic acid.

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