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The effect of endotoxin on plasma α-aminoisobutyric acid[1]

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Summary. The i.v. injection of bacterial endotoxin into dogs was found to cause a rapid increase in plasma levels of infused α-aminoisobutyric acid. The findings suggest that nonmetabolic factors (tissue uptake, fluid shifts) influence amino acid distribution during endotoxemia.

Altered nitrogen utilization and distribution are among the most critical consequences of infection with plasma amino acids generally declining in the afebrile state even before the onset of anorexia[2,3]. The metabolic response to early sepsis may differ from prolonged infection since i.v. injection of live *Escherichia coli* bacteria in dogs produces a rapid and pronounced elevation of alanine and other amino acids[4]. This increase has been attributed to a reduced hepatic conversion of alanine to glucose. The injection of *E. coli* endotoxin into dogs also increases plasma alanine levels but gluconeogenesis from U-^{14}C-alanine was not diminished after 4 h of endotoxemic shock[5]. To determine if nonmetabolic factors are involved in redistributing amino acids in early endotoxemia, 1-^{14}C-α-aminoisobutyric acid (AIB) was infused into endotoxin-treated dogs. This inert amino acid analogue is not incorporated into protein or otherwise metabolized but enters into cells by the same carrier system that transports alanine[6,7]. No physiological action or transmitter function has ever been reported for tracer amounts of AIB; its metabolic inertness and very slow penetrance into brain has been confirmed many times[8].

Materials and methods. Overnight fasted dogs of either sex, 15-20 kg, were anesthetized with Nembutal (30 mg/kg) and infused with 1-^{14}C-α-aminoisobutyric acid (AIB) (Amersham-Searle, Arlington Heights, IL.) in sterile saline at the rate of 0.58 ml/min. Each ml contained 0.15-0.20 mCi of AIB plus 0.66 mg added carrier AIB. An LD_{70} dose of *E. coli* endotoxin (Difco, Detroit, MI) was given i.v.

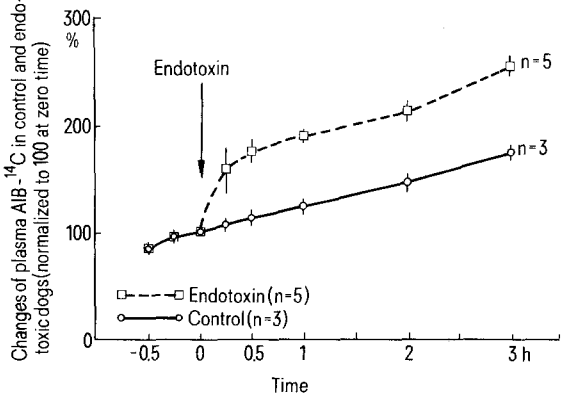

Fig. 1. The increase of plasma levels of infused α-aminoisobutyric acid after endotoxin injection. Vertical lines, mean ± SEM.

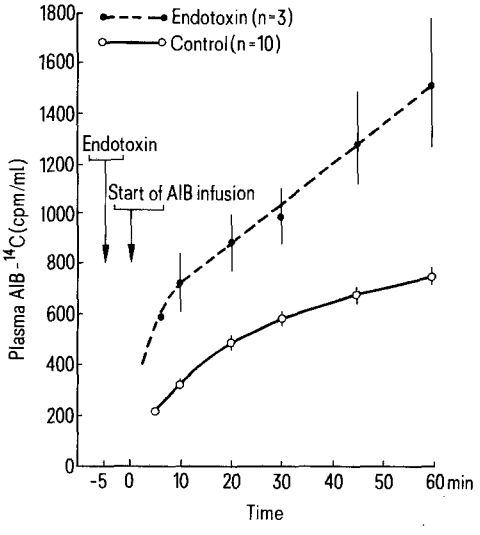

Fig. 2. The increase in plasma counts of α-aminoisobutyric acid when dogs are pretreated with endotoxin before AIB infusion. Vertical lines, mean ± SEM.

(0.5 mg/kg). Mean arterial blood pressure was monitored by a Statham pressure gauge. Heparinized plasma samples were deproteinized with perchloric acid and neutralized with 4 N potassium hydroxide. Supernates were counted in a Beckman scintillation counter. Diluted urine was counted directly in Aquasol (New England Nuclear, Boston, MA). Plasma counts were normalized by a factor based on infused radioactivity per kg dog weight.

Results. Figure 1 shows that after plasma AIB counts are reasonably constant following 60–90 min of infusion, the injection of endotoxin rapidly raises AIB levels for the duration of the experiment. This response is similar to the plasma alanine increase produced by *E. coli* endotoxin[5]. Figure 2 indicates that plasma AIB counts are higher at every sample interval when endotoxin is injected prior to the start of the AIB infusion.

Endotoxin consistently lowers arterial blood pressure so that renal filtration is curtailed. 3 control dogs with urethral catheters were infused with AIB for 30 min to assess the significance of this renal effect. An average of 2.7% (2.2, 2.3 and 3.5%) of the total infused AIB or about 117,000 cpm were recovered from the urine after several bladder washes. If total renal shutdown occurs in endotoxified dogs, adding these urine counts to an estimated 800 ml plasma and 1600 ml extracellular fluid (ECF) would raise control fluid (plasma plus ECF) only 49 cpm/ml at the 30-min interval. The difference between control and endotoxemic plasma at this sample time is 400 cpm/ml. Thus the maximum contribution urinary AIB can make to plasma counts is 12% of the observed difference and this assumes complete renal shutdown. Lymph collected from the thoracic duct of one dog approximated plasma counts as expected.

Discussion. Plasma levels of a metabolically inert amino acid infused into dogs are rapidly elevated after endotoxin administration. Plasma alanine has been reported to show a similar response to endotoxin[5]. The present findings suggest that nonmetabolic factors may be operating in the partitioning of both amino acids during endotoxemia. A renal factor seems to be minimal in the AIB experiments.

Membrane damage mediated by endotoxin does not appear to be involved in the AIB increase in view of the results in figure 2. In the first few min of infusion, tissue AIB pools are not in equilibrium with circulating plasma AIB. Even if endotoxin damaged cell membranes, not enough AIB would be present in tissue pools to escape into circulating fluids to raise plasma AIB counts to the extent shown in figure 2. This suggests that endotoxin blocks tissue uptake of AIB and/or causes major fluid shifts in the body.

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Studies on the transmembrane ion currents in the smooth-muscle cells of the gastric fundus

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Summary. Under voltage-clamp conditions fast Ca^{2+}-inward and early K^+-outward currents were recorded from the smooth-muscle cells of the gastric fundus. It is assumed that the less electrical excitability of these cells is due to the early activation of the outward current.

The smooth muscles of the fundic region of the stomach do not[2] or rarely[3-5] manifest spontaneous electrical and contractile activity. Electrical stimulation does not always lead to the occurrence of regenerative spike potentials[6-11]. Thus some authors[6] suggest the presence of electrically less excitable muscle fibres which determine the blockade or the inhibition of the propagation of excitation in the fundic smooth muscles. Using the voltage clamp method we studied the membrane ion currents with the purpose of elucidating the causes of the slight electrical excitability of the fundic smooth-muscle cells.

Materials and methods. Circular muscle strips, 0.2–0.3 mm wide and 10–12 mm long, were removed from the fundus of the guinea-pig stomach. The strips were kept in Krebs solution for 1–2 h and were then placed in a chamber with a double sucrose gap, whose test compartment was 0.4 mm wide. The experiments were carried out under current clamp and voltage clamp conditions[7] in normal Krebs solution at 36 °C. The Na-free solutions contained choline chloride instead of Na in the presence of atropine (10^{-6} M). In some of the experiments Ba^{2+} (2.5 mM) was substituted for Ca^{2+}.

Results and discussion. Polarizing currents applied under current clamp conditions led to the occurrence of electrotonic potentials in the fundic smooth-muscle cells. In some of the preparations a low depolarization was superimposed on the electrotonic potentials resembling local excitation which did not develop into an action potential even at high intensity of depolarizing current (figure 1, a). The current-voltage relation was linear as upon depolarization and the membrane resistance decreased with increasing current intensity (figure 1, c). The time constant of the membrane was 150–180 msec.

Under voltage-clamp conditions we observed a fast inward current which reached its maximum values within 5–10 msec. The development of the fast inward current was interrupted by the early activation of the fast outward current (figure 1, b). The dynamics of the ion currents through the membrane of the fundic muscle cells resembled the dynamics of development of the ion currents from the ureter[8] where the activation of the fast K-outward current was slightly delayed after the activation of the fast inward current. That is why the inward and outward currents overlapped in a wide time interval. This led to a considerable decrease of the resultant transmembrane currents. Unlike the ureter in the fundic muscle cells the fast outward current was greater and in some of the strips it reached such high values that it inhibited the inward