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Colonic Bacteria: A Source of γ-Aminobutyric Acid in Blood (41169)

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Abstract. Blood contains more of the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA), than does cerebrospinal fluid. However, the source and physiologic importance of this compound in the peripheral circulation are unknown. We have found that blood drawn from the portal vein of normal rabbits contains twice the concentration of GABA as that drawn simultaneously from the abdominal aorta. Also, human-derived colonic bacteria grown anaerobically secreted a large amount of GABA equivalents into culture media when measured by a radioreceptor technique. The production of inhibitory neuroactive substances, such as GABA, by colonic bacteria may play a role in the pathogenesis of certain disease states, for example, hepatic encephalopathy.

Many reports have been published confirming the presence of γ-aminobutyric acid (GABA) in blood (1–3). Several of these reports have commented that the source of this compound is unknown, since the enzyme responsible for the generation of GABA (L-glutamate 1-carboxylase, EC 4.1.1.15) is found in very low concentrations outside the central nervous system (4). Some authors have speculated that GABA might reach the peripheral circulation by diffusion from the cerebrospinal fluid (CSF) (1). However, since the concentration of GABA in plasma is higher than in CSF (3), this seems an unlikely explanation. There appears to be agreement, however, that GABA injected peripherally is rapidly removed from the circulation as a result of transamination in the liver and renal cortex, and renal excretion (5, 6). Because GABA occurs as a metabolite in a wide variety of bacteria (7), we have considered the possibility that colonic bacteria could be a source of GABA in blood.

Materials and Methods. *Animals.* Standard New Zealand White rabbits were obtained from the NIH Small Animal Section. They weighed between 2 and 3 kg. Seven such rabbits were anesthetized by the intravenous administration of pentobarbital (A. J. Buck & Son, Cockeysville, Md.). The abdomen was quickly opened and samples of blood taken from the portal vein and abdominal aorta. These samples were allowed to clot and centrifuged. Sera were frozen at −20°.

GABA assay. The concentration of GABA in serum was estimated utilizing a standard radioreceptor assay (1). Neural membranes enriched in synaptic regions were prepared from normal rabbit brain by the method of Zukin *et al.* (8). These membranes were frozen at −20° for at least 18 h before use in order to enhance postsynaptic binding and reduce presynaptic binding (9). The frozen suspension was thawed and Triton X-100 was added to yield a 0.05% (v/v) concentration of detergent. The suspension was incubated at 37° for 30 min and then centrifuged at 48,000*g* for 20 minutes at 4°. The supernate was decanted and the pellet resuspended in 0.05 *M* Tris–citrate buffer (pH 7.1). This wash was repeated three times. The final pellet was resuspended to a protein concentration of 1 mg/ml. Two hundred microliters of this suspension was incubated with 100 μl of sample, 100 μl of [^{3}H]GABA (New England Nuclear Co., 40 Ci/mmole) solution (12.6 pmole of [^{3}H]GABA per 100 μl) and 600 μl of 0.05 *M* Tris–citrate buffer (pH 7.1) for 5 min at 4°. The mixture was then centrifuged at 48,000*g* for 10 min. The resulting pellet was rapidly washed with iced buffer and dissolved in Protosol (New England Nuclear Co.). Radioactivity present in the dissolved pellet was estimated by scintillation counting using Ultrafluor as the

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counting solution and a Mark II counter (Nuclear-Chicago). A standard curve was generated for each series of test sera, by substituting eight different known amounts of unlabeled GABA for test samples in the above assay. Standard curve determinations were run in duplicate. Test specimens were assayed in triplicate. In this system, about 20,000 cpm represents maximal binding. In the presence of 1 m*M* unlabeled GABA binding of [^{3}H]GABA falls to about 5000 cpm.

Microbiology. Three species of human colonic bacteria (*Bacteroides fragilis, Escherichia coli, Peptostreptococcus*) were isolated and cultured by the Anaerobe Laboratory of the Clinical Microbiology Section of the NIH Clinical Center Clinical Pathology Laboratory. All cultures were incubated under anaerobic conditions for 4 days. Nutrient broth (Difco) was used as basal medium for *B. fragilis* and *E. coli.* Brain–heart infusion (Difco) was used as a basal medium for the more fastidious *Peptostreptococcus.* At the end of the incubation period growth of the cultures was estimated by a clinical microbiologist. Aliquots (7 ml) of the cultures were centrifuged at 3000*g* for 20 min and supernates frozen at −20° for analysis the next day. As the specificity of the assay for GABA has not been demonstrated in bacterial systems, results are expressed as GABA equivalents.

Sample preparation. The sensitivity of the method required extensive sample dilution. Sera were diluted 1:100 or 1:1000 with triple-distilled water before assay. Supernatants of bacterial cultures after centrifugation were serially diluted up to 1:100,000 before assay.

Statistics. GABA concentrations of portal and aortic blood samples were compared by paired *t* test.

Results. Serum GABA concentrations of samples simultaneously obtained from the abdominal aorta and portal vein differed in each of the seven rabbits tested, with the portal concentration always being higher. The mean aortic concentration was 499 ± 130 (SD) pmole/ml and the mean portal vein concentration was 954 ± 160 pmol/ml. This was a statistically significant difference ($p < 0.005$). Thus, the bowel contributes GABA to the circulation.

Table I shows the concentration of GABA equivalents in supernates of the bacterial cultures. Production of GABA equivalents by bacteria paralleled the luxurience of growth in cultures of *B. fragilis* and *E. coli. Peptostreptococcus,* however, reduced GABA equivalents in the culture medium by a small amount. Thus, two species of bacteria which are present in the colon in large numbers are capable of generating large amounts of GABA, while at least one other species, which is found in the colon in relatively smaller numbers may be able to catabolize it.

Discussion. The results of these studies provide support for the hypothesis that bacteria in the large bowel contribute to GABA in blood. The colon of man contains large numbers of bacteria, the vast majority of which are fastidious anaerobes. *B. fragilis,* for instance, is present in a concentration of 10^9 to 10^{12} per gram of intesinal contents (10). Also, the colon is capable of absorbing amino acids (11). We have shown that, *in vivo,* blood leaving the gut contains higher concentrations of GABA than blood perfusing the gut from the aorta. We have also shown that the major bacterial residents of the colon are capable of generating large quantities of GABA when grown anaerobically. The simplest

TABLE I. PRODUCTION OF GABA EQUIVALENTS BY CULTURED ANAEROBIC BACTERIA (pmole/ml SUPERNATANT)

Growth	*B. fragilis*	*E. coli*	*Peptostreptococcus*
++++		24,725	−1750
+++	33,795	13,095	
++	4,075	55	
+	279	30	

Note. Each number represents either a single culture or the average of up to five cultures which grew at the given rate. No number is given if 4-day cultures at that rate were not obtained. Each culture was incubated with an uninoculated control. The assayed GABA content of the control was subtracted from the assayed GABA content of the experimental culture to yield each data point. Very few GABA equivalents were found in the uninoculated controls for the *B. fragilis* and *E. coli* (about 10 pmole/ml) but the controls for the *Peptostreptococcus* contained appreciable amounts (13,000 pmole/ml) as might be expected as the media was a brain–heart infusion.

interpretation of these data is that GABA is produced by colonic bacteria, absorbed from the colon, and thereafter removed from the circulation by known mechanisms in liver and kidney. As GABA does not normally cross the blood–brain barrier (12), this cycle of synthesis, degradation, and excretion of GABA could operate independently from GABA metabolism in the central nervous system. Such independence of CNS and extra-CNS GABA metabolism would help to explain why other workers have been unable to find any constant relationship between plasma and CSF levels of GABA (3).

The results of this study are also of potential pathophysiologic relevance. The blood–brain barrier is known to become more porous in certain disease states, specifically hepatic encephalopathy (13). Severe hepatic disease causing a decrease in functional liver cell mass and/or shunting of blood from portal to systemic circulation might result in decreased hepatic clearance of colon-produced GABA. The combination of increased porosity of the blood–brain barrier and decreased clearance of GABA by the liver could result in increased transfer of colon-produced GABA into the CNS. There, GABA might act to mediate the generalized neural inhibition of hepatic encephalopathy.

Purgation, oral nonabsorbable broad spectrum antibiotics, reduced protein intake, and colonic exclusion have long been used as treatments of portal-systemic encephalopathy. The success of these treatments in ameliorating hepatic encephalopathy has led to the suggestion that neuroactive substances produced by enteric bacteria play an important role in the pathogenesis of hepatic coma. GABA deserves consideration in this respect.

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