

Gamma-Aminobutyric Acid (GABA) and Sepsis-Related Encephalopathy

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ABSTRACT: In order to determine whether disturbances in GABA homeostasis might play a role in the pathogenesis of sepsis-related encephalopathy, serum and brain tissue GABA concentrations from six areas of the brain (cortex, diencephalon, striatum, hippocampus, midbrain, and pons-medulla) were determined in a rat model of bacterial sepsis (cecal ligation and perforation). The results were compared to those obtained from sham operated control animals. All septic animals demonstrated clinical signs of encephalopathy and had elevated serum GABA levels ($0.92 \pm 0.3 \mu\text{M}$ versus 0.48 ± 0.15 in controls, $p < 0.01$). GABA content in the specific subcompartments of the brain, however, were similar in the two groups. These results indicate that although serum GABA levels are elevated during sepsis, GABA is unlikely to play an important role in the pathogenesis of sepsis-related encephalopathy.

RÉSUMÉ: L'acide gamma-aminobutyrique (GABA) et l'encéphalopathie en rapport avec la septicémie. Nous avons déterminé les concentrations de GABA dans le sérum et le tissu cérébral de six régions du cerveau (cortex, diencephale, striatum, hippocampe, mésencéphale et pont) chez un modèle animal de septicémie bactérienne (rat ayant subi une ligature caecale avec perforation) afin de déterminer si des altérations de l'homéostasie pouvaient jouer un rôle dans la pathogénèse de l'encéphalopathie survenant en relation avec une septicémie. Les résultats ont été comparés à ceux obtenus chez des animaux contrôles ayant subi une intervention factice. Tous les animaux septicémiques présentaient des signes cliniques d'encéphalopathie et avaient des taux sériques élevés de GABA ($0.92 \pm 0.3 \text{ M}$ versus 0.48 ± 0.15 chez les contrôles, $p < 0.01$). Le contenu en GABA des différents sous-compartiments du cerveau était cependant semblable dans les deux groupes. Ces résultats indiquent que, même si les taux sériques de GABA sont élevés pendant la septicémie, le GABA ne joue vraisemblablement pas un rôle important dans la pathogénèse de l'encéphalopathie survenant en relation avec une septicémie.

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Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain. Recently, a series of reports have implicated GABA in the pathogenesis of certain forms of metabolic encephalopathy.¹⁻¹¹ In particular, elevated serum GABA levels were significantly higher in patients with hepatic encephalopathy than in those with liver disease and no evidence of encephalopathy.^{3,4,7} Elevated serum GABA levels were also observed in patients with chronic renal failure.⁴ Although patients in the latter study were not assessed for evidence of encephalopathy, the possibility was raised that GABA might play a role in the pathogenesis of uremic encephalopathy. Finally, low CSF and brain GABA concentrations were reported in six adult patients with dialysis encephalopathy.¹⁰

A depressed level of consciousness is a frequent finding in patients with bacterial sepsis.¹²⁻¹⁴ The explanation for this finding remains unclear. Recently, it has been demonstrated that aerobic and anaerobic bacteria are capable of producing large amounts of GABA *in vitro*.^{15,16} To determine whether

bacterial GABA production might play a role in the depressed levels of consciousness seen with sepsis, we measured serum and brain tissue GABA levels in an animal model of sepsis,¹⁷ and compared the results to sham operated controls.

MATERIALS AND METHODS

A total of 15 adult male Sprague-Dawley rats were subjected to laparotomy. Nine rats underwent cecal ligation and puncture with a #18 gauge needle. Six rats had bowel manipulation but no ligation or puncture. The abdomens were then closed and the rats allowed food and water ad lib. Twenty hours post-operatively, blood samples were obtained by intracardiac puncture and the rats sacrificed by decapitation under ether anaesthesia. Two of the septic rats died prior to decapitation and were not available for study. All study rats demonstrated evidence of sepsis at the time of sacrifice (piloerection, abnormal righting reflex and/or lethargy) while control animals behaved

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normally. Whole brains were immediately excised and samples from six specific regions (cortex, diencephalon, striatum, hippocampus, midbrain, and pons-medulla) of the brain were isolated and frozen, initially in liquid nitrogen and then at -70 degrees Celsius.

GABA analyses on serum were performed by ion exchange fluoremetry as described by Hare and Manyam¹⁸ and on homogenates of the brain as described by Freund et al.¹⁹

A student's tests for unpaired data was used for statistical analyses. The null hypothesis was rejected if the value of p was less than 0.05.

RESULTS

Serum GABA levels in septic rats (0.92 ± 0.03 μM , mean \pm SD) were significantly higher than in controls (0.48 ± 0.15 μM , $p < 0.01$).

Although brain tissue GABA levels tended to be higher in all areas of the brain of septic animals, the differences failed to reach statistical significance (Figure 1).

DISCUSSION

An altered level of consciousness is a well-recognized manifestation of severe sepsis.¹²⁻¹⁴ The mechanism whereby it occurs, however, remains unclear. Plum and Posner²⁰ have suggested that decreased cerebral energy production is important in such encephalopathic states. Fischer et al.,²¹ on the other hand, have implicated the false neurotransmitter hypothesis wherein increased brain uptake of aromatic amino acids (relative to branched chain amino acids) ultimately compete with true neurotransmitters for CNS receptor sites. More recently, Jackson et al.²² suggest that both toxic mechanisms and bacterial invasion of the brain with the formation of disseminated microabscesses are operative.

The role of GABA in the pathogenesis of metabolic encephalopathy has been considered for a number of reasons. Firstly, systemic infusions of GABA agonists are associated with visual evoked potential changes similar to those described in animals and humans with metabolic encephalopathy.⁶ Secondly, changes in brain GABA receptor density have been reported in animal studies of metabolic encephalopathy.^{6,23,24} Thirdly, abnormal serum GABA levels have been documented in patients with

hepatic encephalopathy, advanced renal disease and dialysis encephalopathy.^{3,4,7,10} Fourthly, the administration of benzodiazepine antagonists (which compete with benzodiazepines for GABA receptor sites) has been shown to cause prompt improvement in the encephalopathic state of animals and humans with advanced encephalopathy.^{9,11,25} Finally, bacteria are capable of producing large amounts of GABA and bacterial sepsis is a common precipitating cause of hepatic encephalopathy.^{15,16}

The results of the present study, however, would at best be considered only partially supportive of the GABA hypothesis for sepsis-related encephalopathy. For although serum GABA levels in septic rats were significantly higher than in a comparable control population, it remains to be determined to what extent peripheral GABA gains access to the central nervous system in the presence of an intact blood-brain barrier. Moreover, although brain tissue GABA concentrations were elevated in all six regions of the brains of septic animals; the differences when compared to non-septic controls were not statistically significant.

The finding of elevated serum GABA levels in animals with severe sepsis raises interesting questions regarding other aspects of the septic state. For example, bacterial sepsis is commonly associated with elevated serum catecholamines, cortisol, glucagon, lowered systemic blood pressure and a hyperdynamic circulation.¹² Recent work by this laboratory and others have demonstrated that GABAergic mechanisms appear to be involved in the physiologic control of each of these areas.²⁶⁻²⁸ Further work, however, is required to determine the precise nature of that involvement, particularly in the septic state.

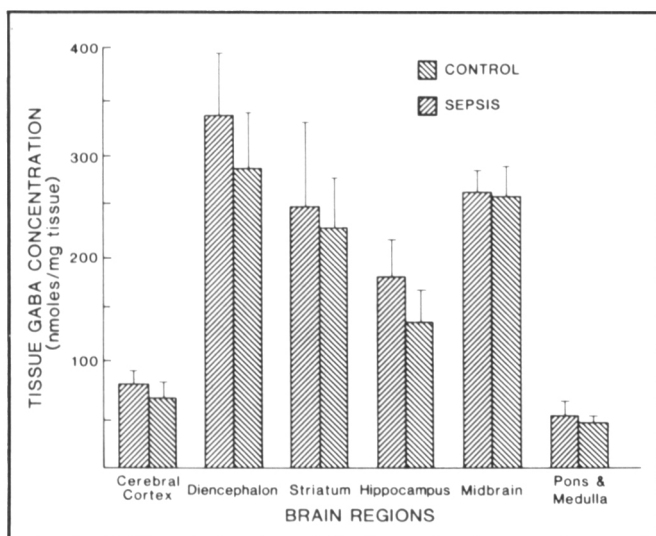
In summary we have shown that in rats with severe sepsis, serum GABA levels are elevated but brain tissue GABA concentrations remain unaltered. Although these preliminary results suggest that GABA is unlikely to play an important role in the pathogenesis of sepsis-related encephalopathy, consideration should be given as to what effect these changes might have on other aspects of bacterial sepsis.

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