Effect of Probenecid on 5-Hydroxyindoleacetic Acid in Cisternal Cerebrospinal Fluid of Rats with Portacaval Anastomosis

Marcelle Bergeron, Margaret S. Swain, Eduardo Molina-Holgado, Tomás A. Reader, and Roger F. Butterworth

(Accepted May 22, 1995)

Portal-systemic encephalopathy (PSE) is characterized by a neuropsychiatric disorder progressing through personality changes, to stupor and coma. Previous studies have revealed alterations of serotonin and of its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in brain tissue and CSF in experimental (rat) and human PSE. Increased brain 5-HIAA concentrations could result from its decreased removal rather than to increased serotonin metabolism. In order to evaluate this possibility, CSF 5-HIAA concentrations were measured using an indwelling cisterna magna catheter technique at various times following end-to-side portacaval anastomosis in rats (the most widely used animal model of PSE) treated with probenecid, a competitive inhibitor that blocks the active transport of acid metabolites out of the brain and CSF. Following portacaval anastomosis and probenecid treatment, CSF concentrations of 5-HIAA were increased to a greater extent than in sham-operated controls. When data were expressed as per-cent baseline values, the relative increase of CSF 5-HIAA in portacaval shunted rats following probenecid treatment was not significantly different from sham-operated controls. These findings confirm that increased 5-HIAA in the CNS in experimental PSE results from increased 5HT metabolism or turnover and that the probenecid-sensitive acid metabolite carrier is intact in PSE.

KEY WORDS: Portacaval anastomosis; hepatic encephalopathy; serotonin; 5-hydroxyindoleacetic acid; probenecid; monocarboxylic acid transport.

INTRODUCTION

Portal-systemic encephalopathy (PSE) resulting from chronic liver disease and/or portacaval anastomosis (PCA) in humans is associated with a spectrum of neuropsychiatric symptoms including disorientation, personality changes, loss of memory, sleep and circadian rhythm disorders progressing to stupor and ultimately deep coma (1). Similarly, the creation of an end-to-side PCA in the rat results in altered sleep patterns and circadian rhythms as well as in behavioral abnormalities (2–4). The biochemical mechanisms underlying the pathogenesis of PSE have not been definitively established but accumulating evidence suggests that a defect of serotonin (5HT)-mediated neurotransmission could be responsible for some of the neuropsychiatric symptoms. In both human and experimental PSE, concentrations of tryptophan have consistently been found to be increased in plasma, brain and cerebrospinal fluid (CSF) (5–7).
Studies in humans (8,9) and rats following PCA (2,6,10) have revealed either increased or unchanged brain tissue levels of 5HT. Increased concentrations of the 5HT metabolite 5-hydroxy indoleacetic acid (5-HIAA) have consistently been reported in CSF from rats with PCA (3,10) as well as in humans with chronic liver failure (5,11).

Changes in the concentrations of 5-HIAA in brain have been widely used as indicators of changes in 5HT turnover. Numerous studies in both portacaval shunted rats (6) as well as in human PSE (8,11) have afforded evidence of increased brain 5HT turnover as reflected by increased 5HIAA concentrations and increased 5-HIAA/5HT concentration ratios (6,8,11). However, increased levels of 5-HIAA in brain and CSF could also be the consequence of increased oxidative metabolism or decreased metabolite removal from brain and CSF. The elimination of acidic metabolites such as 5-HIAA occurs by active transport at the choroid plexus and the blood-brain barrier (12). Probenecid [p-(diisopropylsulfamoyl) benzoic acid] competitively inhibits this active transport from the brain and CSF compartments to the blood compartment without interfering with the mechanisms of synthesis and degradation of 5HT (13,14). In the present study, we determined the time-course of 5-HIAA increases in CSF after blockade of the exit transporter by probenecid in rats with PCA. To determine the time course of 5HT disturbances in PSE and since many changes in 5HT metabolism have been reported as early as 1 day after PCA in the rat (6), the study was carried out at 1 day, 2 weeks and 4 weeks following PCA.

**EXPERIMENTAL PROCEDURE**

Thirty six male Sprague-Dawley rats (200-225 g) were anesthesitized with isoflurane and an end-to-side portacaval anastomosis (PCA) was constructed (n = 18) or a sham operation (n = 18) performed as previously described (15). The latter consisted of a laparotomy and was constructed (n = 18) or a sham operation (n = 18) performed as previously described (15). Animals were then allowed to recover with free access to food and water for periods of 1 day (n = 6), 2 weeks (n = 6) or 4 weeks (n = 6). Two days before CSF sampling and probenecid administration, rats were implanted with a permanent cannula in the cisterna magna following a procedure which allows for repeated sampling of CSF in freely-moving rats (16,17). Animals were anesthetized with isoflurane and placed in a stereotaxic frame. After incision and retraction of the skin, a Burr hole was drilled in the skull on the sagittal midline, 1-2 mm anterior to the interparietal-occipital crest. A catheter, constructed from polyethylene tubing (PE10), was implanted via a cranial approach into the cisterna magna and secured in place with dental acrylic cement. Correct catheter placement was confirmed by the appearance of a clear CSF outflow. Rats were sutured and allowed to recover in individual cages with free access to food and water. The whole operation took approximately 20 min and the procedure was well tolerated by all animals. Following a brief (3-5 day) period of periooperative weight loss, animals gained weight normally and showed no evidence of overt neurological dysfunction.

On the day of the experiment, an initial 25 µl CSF sample was withdrawn from each animal, followed by intraperitoneal (i.p.) injection of probenecid (200 mg/kg dissolved in 1 M sodium hydroxide (NaOH) and made up to volume with 0.1 M phosphate buffer; final pH 7.5). This dose of probenecid was chosen since previous studies have shown that it results in a complete blockade of probenecid-sensitive egress of the monoamine acidic metabolites from the CSF (14,16). No adverse effects and no apparent signs of neurological deterioration were observed after administration of probenecid in either sham or PCA animals. Repeated CSF sampling was subsequently carried out 1, 2, 3, 4, 5, 6, and 24 h after probenecid administration as previously described (16). Samples were immediately frozen at −70°C until analysis. After the last CSF sampling, animals were killed by decapitation and the liver-to-body weight ratio determined as a confirmation of shunt or sham operation. All experimental animal procedures were performed in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Animal Ethics Committee of the André-Viallet Clinical Research Center (University of Montreal).

The levels of 5-HIAA were measured in CSF using reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection as previously described (8,10). Briefly, CSF samples (10-20 µl) were diluted with the mobile phase to a final volume of 75-180 µl. After filtration through a 0.2 µm mesh, the samples were directly injected into the HPLC system. The mobile phase consisted of 0.1 M monochloracetic acid (MCA) adjusted with NaOH to pH 3.30-3.35, containing 800 mg/l of Na₂-EDTA, 460 mg/l of octyl sodium sulfate, and 10-12% (v/v) of HPLC-grade methanol. The flow rate and the temperature of the column were kept at 0.6 ml/min and 32°C, respectively. The glassy carbon working electrode was maintained at a potential of +0.75 V and the gain was set at 20 nA full scale. For each chromatographic run, external standards of 3.125 ng of each compound studied (dissolved in 0.1 M MCA) were assayed in order to determine peak areas as well as retention times. Both parameters showed a very good reproducibility with inter-assay coefficients of variation of less than 5%.

**Statistical Analysis.** Statistical analysis was performed by unpaired Student t test for comparison of data from sham-operated versus portacaval shunted rats. For intergroup comparisons use was made of one-way ANOVA with the post-hoc Tukey multiple comparisons test.

**RESULTS**

As shown in Table I, PCA in the rat resulted in significantly decreased liver and body weights after 2 and 4 weeks in agreement with previous studies (6,18). Though not significant, decreased liver-to-body weight ratio was also observed as early as 1 day after PCA (−9% of controls). The liver-to-body weight ratio decreased further at 2 weeks after PCA (−40%) but remained at a similar level thereafter (−41% after 4 weeks) as previously reported (18).

Baseline CSF concentrations of 5-HIAA from sham-operated control rats (Table II) were in good agreement with previous reports (16,17). Portacaval anastomosis resulted in significant increases of CSF con-