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Long-term cognitive impairment, neuronal loss and reduced cortical cholinergic innervation after recovery from sepsis in a rodent model

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Abstract

Sepsis is a disease with a high and growing prevalence worldwide. Most studies on sepsis up to date have been focused on reduction of short-term mortality. This study investigates cognitive and neuroanatomical long-term consequences of sepsis in a rat model.

Sepsis was induced in male Wistar rats weighing 250–300 g by an i.p. injection of bacterial lipopolysaccharide (LPS, 10 mg/kg). Three months after complete recovery from sepsis, animals showed memory deficits in the radial maze and changes in open field exploratory patterns but unaffected inhibitory avoidance learning. Behavioral findings were matched by sepsis-induced loss of neurons in the hippocampus and the prefrontal cortex on serial sections after NeuN-staining and reduced cholinergic innervation in the parietal cortex measured by immunoradiography of vesicular acetylcholine transporter (VAChT).

Together these results suggest that sepsis can induce persistent behavioral and neuroanatomical changes and warrant studies of the neurological long-term consequences of sepsis in humans.

Keywords: Sepsis; Memory disorders; Behavior; Hippocampus; Rat; Cortex; Cholinergic innervation; Lipopolysaccharides

Introduction

Sepsis is a frequent condition accounting for 2.26 cases per 100 hospital discharges which translates into an annual burden of approximately 750,000 cases in the US population (Angus et al., 2001). An essential feature of sepsis is the rapid production of cytokines, chemokines, prostaglandins and nitric oxide (NO) (Rietschel et al., 1996). The potential neurotoxic effects of these proinflammatory mediators have been well documented (Chao et al., 1995; Hu et al., 1997; Zhao et al., 2001; Reimann-Philipp et al., 2001; Liu et al., 2002). Accordingly, cell death within the central nervous system during sepsis has been described in rodent models and humans (Messaris et al., 2004; Sharshar et al., 2004; Semmler et al., 2005). The inflammatory and immune responses evoked in sepsis may create not only an acute brain dysfunction, septic encephalopathy, which occurs in the majority of septic patients (Pine et al., 1983; Young et al., 1990; Sprung et al., 1990), but possibly also long-term deficits. Nevertheless, only a few investigations have addressed the long-term consequences of sepsis and have lead to partly conflicting results (Perl et al., 1995; Heyland et al., 2000; Granja et al., 2004). Recently, in rats recovered from sepsis induced by cecal ligation and puncture, impaired avoidance learning and habituation patterns, but no changes in basal activity were observed 10 and 30 days after surgery (Barichello et al., 2005a,b). In the present study, we investigated whether sepsis induced by i. p. injection of lipopolysaccharide (LPS) in rats leads to persistent impairment of cognitive capacities and neuroanatomical changes.

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Methods

Animals and animal procedures

Male Wistar rats (Charles River, Sulzfeld, Germany) weighing 250–300 g were housed in groups of four under standard conditions at a temperature of 22 °C (±1 °C) and a 12 h light–dark cycle (light from 06:00 to 18:00) with free access to standard food (Altromin, Soest, Germany) and tap water.

To initiate experimental sepsis, rats ($n=18$) received 10 mg/kg of LPS (0127:B8, *E. coli*; Sigma) dissolved in 1 ml sodium chloride (0.9%) intraperitoneally. Control animals ($n=18$) received the vehicle alone. After the intraperitoneal injection, animals were transferred back to their cages for 12 weeks to ensure complete recovery from sepsis. After this period the behavioral experiments were performed.

Behavioral procedures

Open field

Open field experiments were performed in a silent dimly lit room. The open field was a square acrylic glass box (60 × 60 × 60 cm). A video camera was placed 200 cm above the center of the open field. Illumination density at the enter of the maze was below 1 lx. For data analysis the ground floor of the box was subdivided by 2 × 2 lines in nine equally spaced squares. Open field movements were recorded by computer-aided tracking (Chromotrack, v4.02, San Diego, USA). The following parameters were analyzed: line crossings (horizontal activity), time spent in a 5 cm corridor along the maze walls, time spent in the center square (30 × 30 cm), time spent in the 4 corner squares (10 × 10 cm) of the maze and rearing (standing upright with or without touching the walls with the forepaws). Each trial lasted 5 min. After each trial the maze was cleaned thoroughly to avoid confounding effects by urination and fecal boli.

8-arm radial maze task

The radial maze used consisted of 8 equally spaced, opaque arms (50 cm long, 10 cm wide, 20 cm high) extending from a central octagonal hub (25 cm across). Additionally, a removable octagonal form was in the hub for the first minute of the experiment. Fixed visual stimuli served as extramaze cues throughout radial maze testing. The maze was elevated 50 cm from the floor. Food cups at the end of each arm served as receptacles for the food pellet reinforcers (Research Diets, New Brunswick, USA). Five days before the beginning and during the experiment, a restricted feeding schedule was employed. By reducing the amount of daily available food, body weight of each rat was maintained at 80–90% of the freely feeding level. Water was available ad libitum. Two days before the experiment rats were exposed to the apparatus and the food pellets for 10 min. During data acquisition, rats were placed in the central octagonal form, which was lifted after 1 min, and the rats were allowed to move freely in the maze. A trail continued until the test animal had either entered all 8 arms and consumed the baits or until 10 min had elapsed. The following parameters were considered as criteria for radial maze performance: the number of correct choices in the first 8 chosen arms (entry into an arm that the animal had not previously visited), number of errors (reentry into an arm that the rat had previously visited and number of arms the animal had not entered after 10 min) and number of days to reach the learning criterion (not more than one error on two subsequent days). The radial maze experiment was finished after 14 days.

Passive avoidance test

The passive avoidance apparatus consisted of a lit and a dark compartment (25 × 25 × 25 cm size each) with an electrifiable grid floor. The two compartments were separated by a black partition with a circular doorway in the center. For pretraining, the rats were placed in the lit compartment of the apparatus to let them explore it without receiving an electric shock. On the next day (baseline), rats were placed in the lit compartment, and the latency before entering the dark compartment was recorded. When the rat entered the dark compartment with all four paws, the doorway in the center was closed immediately and a 36 V AC foot-shock was applied (1 mA/4 s). Ten seconds after receiving the electric shock, the rat was removed from the dark compartment and gently placed back into the home cage. The testing trials were performed 24 h and 7 days later. The rat was placed into the lit compartment again and the time until entering the dark compartment was recorded. When the rat did not enter the black compartment within 120 s, the test was terminated and a latency of 120 s was recorded.

Processing of brain tissue and immunohistochemistry

For further analysis, animals were killed by an overdose of thiopentone (50 mg/kg), transcardially perfused with 200 ml heparinized sodium chloride (0.9%) and subsequently fixed with 200 ml containing 10% formaldehyde, 10% acetic acid and 80% methanol. Brains were removed and immersed in the fixative for additional 72 h at room temperature, followed by paraffin embedding using standard protocols. All experiments were carried out in accordance with the animal welfare guidelines and laws of the Federal Republic of Germany and were approved by the local ethical committee.

For immunohistochemistry, 7 μm thick coronal sections of paraffin embedded brain were made using a Leica microtom model 2155 and mounted on poly-L-lysine coated slides.

For NeuN staining, the sections were washed in PBS and incubated in diluted horse serum (1:10 with PBS) for 45 min. Afterwards they were incubated with monoclonal anti-NeuN antibody (Chemicon MAB 377, mouse IgG1, 1:500 in 2% horse serum in PBS) overnight at 4 °C. Thereafter they were treated with a biotinylated second antibody (1:200 in 2% horse serum in PBS) and after washing incubated in PBS with avidin-biotinylated peroxidase complex (Vectostain ABC-Kit, Vector Lab, Linaris Wertheim, Germany).

Quantification of hippocampal, entorhinal and parietal cortex neurons

Quantitative analyses were performed with computer-assisted image analysis consisting of a Nikon Eclipse 800 microscope (Nikon GmBH, Düsseldorf, Germany), a Sony camera (Sony, Model DXC-9100 P, Sony Köln, Germany) and a