Intraventricular infusion of N-methyl-D-aspartate

1. Acute blood-brain barrier consequences*

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Summary. The purpose of this study was to document the early cerebrovascular consequences of excessive N-methyl-D-aspartate (NMDA) receptor activation. Five microliters of NMDA (100 nmol/μl) or vehicle was infused over a 15-min period into the lateral ventricle of adult rats. The protein tracer horseradish peroxidase (HRP) was injected intravenously for blood-brain barrier (BBB) studies. The intraventricular infusion of vehicle (n = 5) caused no alterations in arterial blood pressure or microvascular damage away from the intraventricular probe tract. In contrast, NMDA infusion (n = 8) led to a gradual increase in arterial blood pressure (mean 36 mm Hg). Multifocal regions of HRP extravasation were observed bilaterally throughout the neuraxis following NMDA infusion. Sites of BBB disruption and hemorrhage included brain regions bordering ventricular spaces. In addition, isolated foci of protein extravasation were commonly detected in the cerebral cortex, thalamus, basal forebrain, septum and cerebellum. Pretreatment with the noncompetitive NMDA antagonist MK-801 (2mg/kg) substantially reduced the BBB responses to NMDA. However, microvascular abnormalities were seen in NMDA-infused rats where blood pressure elevations were inhibited by blood removal. In addition to neurons, cerebral blood vessels are also acutely affected by NMDA receptor activation. Blockage of NMDA receptor channels following brain injury may potentially provide protection by attenuating BBB breakdown and subsequent brain edema.

Key words: Blood-brain barrier – Endothelium – Excitotoxicity – N-Methyl-D-aspartate – Electron microscopy

It has long been recognized that neurons are structurally injured when exposed to the excitatory amino acid glutamate [33, 35, 46, 48, 49]. Olney [46] first reported pathological changes in the circumventricular regions of neonatal mice when glutamate was injected systemically at relatively high doses. These structural changes took on a characteristic pattern including neuronal cell body and dendritic swelling (dendrosomatoxic swelling) with axon sparing. In the adult brain, relatively high doses of glutamate are required to produce structural damage [47, 59] due to the limited transport across the blood-brain barrier (BBB) and efficient uptake mechanisms [23, 32, 43].

N-Methyl-D-aspartate (NMDA), kainate (KA) or other potent agonist acting on glutamate receptors also produce neuronal damage characterized as “excitotoxic” when injected into the brain. Excitotoxic mechanisms following NMDA receptor activation have been implicated in various brain injuries including ischemia, trauma, seizures and neurodegenerative diseases [10, 28, 34, 47, 58, 60]. Although neurons contain NMDA receptors, previously published studies have failed to document NMDA receptors on cerebrovascular endothelial cells [5]. In addition, cerebrovascular tone does not appear to be affected by the administration of excitatory amino acids [22]. However, more recent investigations have reported NMDA receptors on isolated rat cerebral capillaries and glutamate-induced endothelial cell degeneration in cell culture studies [17, 30, 31]. Thus, it is conceivable that pathomechanisms of excitotoxic brain injury may involve vascular as well as neuronal components.

Using small variations in brain temperature as a tool to modify ischemic outcome, a relationship between levels of ischemia-induced extracellular glutamate and postischemic BBB disruption has recently been demonstrated. By artificially increasing intraischemic brain temperature to 39 °C, ischemia-induced BBB permeability and levels of extracellular glutamate are significantly increased compared to normothermic conditions [36°–37 °C] [13, 61]. In contrast, decreasing intrais-
chemic brain temperature to 30°–33°C attenuates post-ischemic BBB consequences and the rise in extracellular glutamate [9, 13]. Acute BBB dysfunction following normothermic and hyperthermic brain ischemia may, therefore, involve excitotoxic mechanisms. Consistent with this hypothesis are recent findings demonstrating that BBB disruption can be documented following the infusion of NMDA into cerebrospinal fluid (CSF) spaces of the brain and spinal cord [16, 40].

The major aim of this study was to document the ultrastructural consequences of excessive NMDA receptor activation on cerebral blood vessels and BBB function. NMDA was investigated since excessive levels of extracellular glutamate are difficult to achieve in the normal brain due to uptake systems. NMDA was infused into the lateral ventricle to bypass the BBB and, thus, potentially expose widespread brain areas to the excitotoxin.

Materials and methods

Experiments were performed on 28 male Wistar rats weighing between 250 and 300 g. Anesthesia was initially induced with 3% halothane for 3–5 min and rats were intubated and maintained on 1.5% halothane and a mixture of 70% nitrous oxide and 30% oxygen. Femoral arterial and venous catheters were inserted for measurement of arterial blood pressure, blood gases, and for horseradish peroxidase (HRP) administration. Rats were mounted in a stereotactic frame and soft tissues overlying the right calvarium were retracted laterally. For insertion of the right lateral ventricle probe, a small burr hole was drilled at 1.3 mm posterior to bregma, 1.6 mm lateral and the ventricular probe inserted 3.5 mm ventral to bregma [51]. A thermocouple probe was inserted into the temporals muscle as an indirect measure of brain temperature [8]. Head and rectal temperatures were maintained at 37°C throughout the experimental procedure with a heat lamp placed over the head and body. Rats were allowed to stabilize for a 45-min period before the infusion of phosphate buffer (pH 7.0) or NMDA.

In preliminary studies, the effects of several doses of NMDA (600, 100, 30 and 10 nmol/μl) on BBB disruption were investigated. Although each dose lead to widespread permeability changes, 100 nmol/μl (total 5 μl infused) was investigated in the present study. This concentration of NMDA was chosen since it is comparable to the 1 mM concentration of extracellular glutamate documented following temperature-controlled brain ischemia [19, 20, 61]. This conclusion is based on a dilution factor of 1 to 50 (5 μl NMDA into a total CSF volume of approximately 250 μl) [4], and the reported higher glutamate affinity for NMDA receptors (ten-fold) than NMDA itself [36].

For histochemical studies, 30 mg HRP (Type II Sigma Chemical Company, St. Louis, Mo.) dissolved in 1 ml saline, was injected intravenously in rats pretreated with diphenhydramine hydrochloride (0.5 mg/100 g) at the beginning of the infusion period. Fifteen minutes later, rats were perfused transcardially with 0.9% saline (0.5 mg/100 g) at the beginning of the infusion period. Fifteen minutes later, rats were perfused transcardially with 0.9% saline, 100 μl of HRP (5 gl NMDA into a total CSF volume of approximately 250 μl) [4], and the reported higher glutamate affinity for NMDA receptors (ten-fold) than NMDA itself [36].

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Results

Physiological and control findings

Physiological data from control and experimental rats prior to and during the infusion period are presented in Table 1. Compared to pre-infusion values, a nonsignificant increase in PCO₂ and a significant decrease in PO₂ was documented in the NMDA-infused rats. In addition, a significant rise in arterial blood pressure was also reported in the NMDA-infused rats. Commonly, mild blood pressure elevations were recorded approximately 2 min after the start of the NMDA infusion and reached maximum levels during the next 3 to 4 min. In the other experimental groups, no significant differences between pre- and postinfusion data were demonstrated. In rats where EEG was monitored during NMDA infusion, evidence for definitive seizure activity was not obtained. When EEG activity did change during the infusion period, abnormal neuronal activity in the form of isolated spikes was recorded during the initial minutes of the infusion period.

Reacted Vibratome sections from sham-operated rats which were infused for 15 min with phosphate buffer (vehicle) demonstrated extravasated HRP restricted to cortical areas damaged by the ventricular probe. Reaction product was also occasionally detected lining the wall of the injected (right) lateral ventricle (Table 2; Fig. 1A). Brain regions remote from the probe tract appeared unremarkable by light microscopic examination.

Light microscopic findings following NMDA infusion

Bilateral sites of HRP extravasation were detected in all rats receiving NMDA (Table 2; Figs. 1B–E; 2A–C). Pial vessels appeared extremely sensitive to NMDA infusion with superficial cortical layers appearing electron dense. For example, the anterior cerebral artery within the longitudinal fissure and the posterior branch of the middle cerebral artery within the rhinal fissure commonly displayed extravasated HRP. Large penetrat-