Brain Nitric Oxide Changes After Controlled Cortical Impact Injury in Rats

LEELA CHERIAN, J. CLAY GOODMAN, AND CLAUDIA S. ROBERTSON
Departments of Neurosurgery and Pathology, Baylor College of Medicine, Houston, Texas 77030

Cherian, Leela, J. Clay Goodman, and Claudia S. Robertson. Brain nitric oxide changes after controlled cortical impact injury in rats. J. Neurophysiol. 83: 2171–2178, 2000. Nitric oxide (NO) and the NO end products, nitrate and nitrite, were measured at the impact site after a 5-m/s, 3-mm deformation controlled cortical impact injury in rats. Immediately after the impact injury and the NO and microdialysis probes could be replaced, there was an increase from baseline in NO concentration of $83 \pm 16$ (SE) nM, compared with $0.5 \pm 4$ nM in the sham injured animals ($P < 0.001$). This marked increase in NO occurred at the time of the initial rise in blood pressure (BP) and intracranial pressure (ICP) in response to the injury. After the initial increase in BP and ICP, the BP decreased and stabilized at a value which was $\sim 20$ mmHg below the preinjury values, and ICP plateaued at an average value of $20$ mmHg, compared with $8$ mmHg in the sham-injured animals. This provided an average cerebral perfusion pressure of $40–50$ mmHg, compared with $65–75$ mmHg for the sham-injured animals. These values were relatively constant for the remainder of the 3-h monitoring period. The NO values also stabilized during this time period. By 1 h after the impact injury the NO concentration measured directly using the NO electrode had decreased from baseline values by an average value of $25 \pm 6$ nM. NO concentration remained significantly lower than baseline values throughout the remainder of the 3-h monitoring period. The concentration of nitrate/nitrite in the dialysate fluid also decreased by an average value of $341 \pm 283$ nM 20–40 min after the injury. Dialysate nitrate/nitrite concentrations remained less than the preinjury baseline values throughout the remainder of the 3-h monitoring period. Preinjury treatment with L-nitro-arginine methyl ester (L-NAME) blunted the injury-induced increase in NO and resulted in more severe immediate intracranial hypertension and more severe systemic hypotension at one hour after injury. Mortality was also $67\%$ with L-NAME pretreatment, compared with $1\%$ in untreated animals.

INTRODUCTION

Nitric oxide (NO) is synthesized from L-arginine by a group of isoenzymes named NO synthases. Three isoforms of NO are known, two constitutive isoforms [neuronal NOS (nNOS, type I) and endothelial NOS (eNOS, type III)] and one inducible isoform (iNOS, type II) (Bredt and Synder 1990; Moncada et al. 1991; Pollock et al. 1991). nNOS is expressed in the population of neurons throughout the CNS, in perivascular nerves, and in glia (Bredt et al. 1991; Murphy et al. 1993; Nozaki et al. 1993). NO produced by nNOS is thought to mediate synaptic plasticity and neuronal signaling (Garthwaite 1991) and is important in glutamate release (Kano et al. 1998). NO produced by nNOS also plays a role in the regulation of cerebral blood flow (CBF), particularly in the coupling of CBF and metabolism and in the CBF response to hypercapnia (Chollet et al. 1997; Iadecola and Zhang 1996; Ma et al. 1996a,b; Okamoto et al. 1997). eNOS is expressed in the endothelial layers of large vessels (Faraci 1991). NO produced by eNOS may play a role in resting CBF and inhibit platelet aggregation and leukocyte adhesion (Kubes et al. 1991; Ma et al. 1996b).

A number of differences between the three isoforms of NOS exist. The constitutive isoforms have physiological roles in the regulation of CBF and in neuronal signaling, but iNOS is produced only under pathological conditions. The constitutive isoforms synthesize NO only when intracellular calcium concentration is elevated and calmodulin is bound to the enzyme. In contrast, iNOS produces NO continuously and independently of intracellular calcium concentrations.

Although NO metabolism has been studied extensively after cerebral ischemia, relatively few studies have been published regarding NO metabolism after traumatic brain injury. The studies that have examined the effect of inhibition or stimulation of NOS immediately after injury have given confusing results with both adverse and protective effects being attributed to NO (DeWitt et al. 1997; Mesenge et al. 1996; Wada et al. 1997, 1998a,b, 1999). The purpose of this study was to measure the concentration of NO in the brain after traumatic brain injury by two techniques; directly with a NO electrode and indirectly by measuring the extracellular concentrations of NO metabolic products using the microdialysis technique.

METHODS

Anesthesia and surgical preparation

The study protocol was approved by the institutional animal protocol review committee, using guidelines for humane care and use of animals developed by the National Institutes of Health. Long–Evans rats, weighing 300–400g and fasted overnight, were anesthetized with 3.5% isoflurane in 100% oxygen in a vented anesthesia chamber. After endotracheal intubation with a 16-gauge Teflon catheter, the rats were mechanically ventilated with 2% isoflurane in 100% oxygen for the impact and 3 h monitoring period. A 22-gauge catheter was placed in the tail artery to monitor arterial blood pressure (BP) and to draw blood samples for blood gases, blood glucose, and lactate concentrations. Rectal temperature was maintained at 36.5–37.5°C by a heating pad which was controlled by a rectal thermistor. Brain temperature was monitored with a thermocouple microprobe placed in the brain parenchyma and controlled by a heating lamp directed at the head.

Production of brain injury

The details of the methods to produce the impact injury have been described previously (Hu et al. 1994) The head of the rat was fixed in...
Measurement of NO

NO was measured for 4 h (1 h of baseline and 3 h after injury) by two methods, an NO electrode and a microdialysis technique.

NO ELECTRODE METHOD. NO was measured using NO electrodes (tip diam 200 μm; ISO-NOP200, World Precision Instruments, Sarasota, FL) inserted into the brain at a depth of 1.5 mm at the center of the impact site. The measurement principle of this type of electrode is the oxidation of NO at a working electrode which is kept at a constant potential of 0.85 V against an Ag/AgCl reference electrode (Taha et al. 1992). Selectivity to NO was maintained by a gas permeable membrane covering the electrode. The NO electrode was tested for any cross sensitivity for gases such as N₂, O₂, CO, and CO₂, or biological substances such as nitrite, dopamine, and L-arginine. The redox current proportional to tissue NO concentration was measured with an ISO-NO meter (World Precision Instruments). NO electrodes were calibrated before and after each experiment by a standard method of chemical degeneration of NO using S-nitroso-N-acetyl-D,L-pencillamine (SNAP) and copper sulfate at 37°C. SNAP (RSNO) decomposes to NO and a disulfide byproduct according to the following equation

\[ 2RSNO \rightarrow 2NO + RS - SR \]

Brain NO concentration was calculated from the current measured with the probe positioned in the brain by means of the in vitro calibration curve. Changes in brain NO were expressed by the change in concentration (nM) from baseline values. The response of the electrode to oxygen concentration was a particular concern in this model because oxygen becomes depleted at the contusion site after the impact injury. In vitro calibration curves at room air and at zero oxygen concentrations.

FIG. 1. Nitric oxide (NO) calibration curves oxygen concentrations of room air (pO₂ = 150 mmHg) and at zero oxygen concentrations.

To perform the impact injury, a stereotaxic frame by ear bars and incisor bar. A craniotomy (10-mm diam) was performed on the right side of the skull over the parietal cortex. The impactor tip (8 mm diam) was centered in the craniotomy site perpendicular to the exposed surface of the brain at an angle of ~45° to the vertical. The impactor was adjusted to produce an impact with a velocity of ~5 m/s, a duration of ~130 ms, and a brain deformation of 3 mm.

Experimental groups

Animals were assigned to one of three experimental groups: 1) 5 m/s, 3-mm deformation controlled cortical impact injury (n = 12); 2) sham impact injury (n = 16), which consisted of craniectomy plus placement and removal-replacement of probes as in the injured group; and 3) L-NAME (30 mg/kg) administration 30 min before 5 m/s, 3-mm deformation controlled cortical impact injury (n = 6).

FIG. 2. Changes in NO after administration of L-NAME 30 mg/kg (n = 3).
**Statistical analysis**

Data were summarized as mean ± SE when normally distributed and by median (interquartile ranges) when the distribution was not normal. The NO and dialysate nitrate/nitrite data were analyzed by repeated measures of analysis of variance (ANOVA), using Tukey’s test when multiple comparisons were done. The dialysate amino acid data were analyzed by Mann–Whitney rank sum test.

**RESULTS**

Before the impact injury, physiological parameters including arterial blood gases, blood pressure, intracranial pressure, and brain and rectal temperature were comparable in the three experimental groups (Table 1). The changes induced by the impact injury, compared with a sham injury, are summarized in Fig. 4 and discussed below as immediate (<5 min postimpact) responses and early (5 min to 3 h postimpact) responses. The effects of pretreatment with L-NAME, compared with the untreated injury group, are summarized in Fig. 5. Only data from the first hour postinjury is shown in this graph because most (>67%) of the L-NAME treated animals died before the end of the 3-h monitoring period. The P values for the repeated measures analysis of these comparisons are shown Tables 2 and 3, respectively.

**Immediate postinjury changes in BP, intracranial pressure, and brain NO concentrations**

Within seconds of the cortical impact injury, mean BP increased dramatically to an average value of 189 ± 10 mmHg. With the increase in BP, intracranial pressure (ICP) also increased to an average value of 29 ± 6 mmHg at the peak of the BP spike. The rise in BP was transient and within 1 min of the impact injury fell rapidly to levels that were <20 mmHg below preinjury baseline values. BP remained at this low value for the remainder of the 3-h monitoring period. Although the BP was decreasing, the ICP continued to increase to a peak value of 38 ± 2 mmHg and gradually declined, although remaining well above preinjury values throughout the 3-h period of monitoring.

Immediately after the impact injury and the NO and microdialysis probes could be replaced, there was an increase from baseline in NO concentration of 83 ± 16 nM, compared with 0.5 ± 4 nM in the sham injured animals (P < 0.001). Nitrite/nitrate concentrations in the dialysate did not reflect the increase in NO that was measured directly with the electrode, probably because of the transient nature of the changes in relation to the sample time of 20 min. Although the nitrate/nitrite concentrations did increase in the dialysates in 4 of 10 animals, the average nitrate/nitrite concentration for the group did not significantly change during the first 20 min after the impact injury.

Because the NO was rapidly decreasing at the time that the NO probe was replaced, it is likely that the value observed at the time of the reinsertion was not the peak value that occurred after injury. Neither the peak ICP during the impact (which is a measure of injury severity), the increase in BP, nor the increase in ICP were closely related to that value of NO. The timing of the decrease in NO after the impact injury appeared to be similar to the decrease in BP and therefore in cerebral perfusion pressure.

<p>| TABLE 1. Physiological parameters at baseline and at the end of the experiment |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Weight (gm)</th>
<th>Sham Injury</th>
<th>Impact Injury, untreated</th>
<th>Impact Injury, L-NAME pretreated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>360 ± 25</td>
<td>369 ± 29</td>
<td>360 ± 11</td>
<td>0.714</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>87 ± 2.1</td>
<td>88 ± 3.4</td>
<td>83 ± 1.4</td>
<td>0.870</td>
</tr>
<tr>
<td>Intracranial pressure (mm Hg)</td>
<td>8 ± 1.0</td>
<td>8 ± 1.1</td>
<td>9 ± 1.1</td>
<td>0.998</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td>212 ± 22</td>
<td>201 ± 20</td>
<td>208 ± 6.1</td>
<td>0.765</td>
</tr>
<tr>
<td>pCO2 (mm Hg)</td>
<td>33 ± 1.7</td>
<td>34 ± 2.6</td>
<td>36.4 ± 1.1</td>
<td>0.486</td>
</tr>
<tr>
<td>pH</td>
<td>7.33 ± 0.01</td>
<td>7.34 ± 0.02</td>
<td>7.35 ± 0.02</td>
<td>0.749</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>5.6 ± 0.4</td>
<td>5.4 ± 0.2</td>
<td>0.282</td>
<td></td>
</tr>
<tr>
<td>Blood lactate (mmol/L)</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.06</td>
<td>0.996</td>
<td></td>
</tr>
<tr>
<td>Brain temperature, °C</td>
<td>37.2 ± 0.08</td>
<td>37.3 ± 0.006</td>
<td>37.1 ± 0.14</td>
<td>0.487</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>37.1 ± 0.1</td>
<td>37.1 ± 0.1</td>
<td>0.386</td>
<td></td>
</tr>
</tbody>
</table>
When L-NAME was given before the impact injury, the preinjury BP was significantly higher than in the untreated animals (Fig. 5) and brain tissue NO concentrations tended to be lower. The immediate injury-induced increase in BP and in NO were significantly blunted in the L-NAME treated animals. However, the immediate injury-induced increase in ICP was significantly greater in the L-NAME treated animals.

Early (5 min to 3 h) changes in BP, ICP, and brain tissue NO concentrations

Following the rapid changes in BP and ICP that occurred in the first few minutes after the impact injury, the BP stabilized at a value which was $\sim$20 mmHg below the preinjury values and ICP plateaued at an average value of 20 mmHg. This provided an average cerebral perfusion pressure of 40–50 mmHg. These values were relatively constant for the remainder of the 3-h monitoring period.

The NO values also stabilized during this time period. By 1 h after the impact injury, the NO concentration measured directly using the NO electrode had decreased from baseline values by an average value of 25 ± 6 nM. NO concentration remained significantly lower than baseline values throughout the remainder of the 3-h monitoring period. The concentration of nitrate/nitrite in the dialysate fluid also decreased by an average value of 341 ± 283 by 20–40 min after the injury. Dialysate nitrate/nitrite concentrations remained less than the preinjury baseline values throughout the remainder of the 3-h monitoring period. The decrease in NO and dialysate nitrate/nitrite after the impact injury were similar in magnitude to the decreases observed after administration of L-NAME (30 mg/kg) (Fig. 2).

The changes in the dialysate concentrations of excitatory amino acids and in the amino acids related to NO metabolism are shown in Fig. 6. The postinjury values were analyzed in a pooled sample of dialysate collected during the first hour postinjury, when the nitrate/nitrite concentrations were lowest. The dialysate glutamate concentration increased from 35 ± 11 to 124 ± 40 μM/l after the impact injury. Aspartate concentration increased from 4 ± 1 to 12 ± 5 μM/l. Arginine and ornithine concentrations did not significantly change after the impact injury, but citrulline concentration increased more than eightfold, from 5 ± 2 to 32 ± 15 μM/l.

**Discussion**

Hemodynamic response to traumatic brain injury

The hemodynamic response to traumatic brain injury can be divided into several characteristic phases; immediate, early, and late. The immediate response to the cortical impact injury has been described previously in detail (Cherian et al. 1994; Dixon et al. 1991) and consists of transient hypertension followed by hypotension. An increase in ICP accompanies the hypertension but persists even as the blood pressure decreases. The immediate response is similar in other experimental mod-
els of traumatic brain injury including fluid percussion injury and weight drop models (Marmarou et al. 1994; Yuan et al. 1990). The mechanism of the transient systemic hypertension has been related to a massive sympathetic discharge (Rosner et al. 1984). This biphasic pattern of hemodynamic changes is rarely observed in human head injury, probably because of the transient nature of the findings.

The early response to severe brain injury is characterized by intracranial hypertension, systemic hypotension, and a reduction in CBF. At least part of the low CBF observed during this period is a consequence of a decreased cerebral perfusion pressure (CPP). However, CPP is not low enough to explain the 50% reduction of CBF that occurs (Cherian et al. 1994) and CBF remains low even when BP is restored to normal (Zhuang et al. 1992). A similar pattern of hypoperfusion has been observed during the first few hours after head injury in humans. Bouma et al. (1992) have observed a regional CBF (rCBF) <18 ml/100 g/min in 37% of patients during the first 6 h after traumatic brain injury. Marion et al. (1991) described rCBF values <18 ml/100 g/min in areas surrounding contusions. Martin et al. (1997) described an evolving pattern for CBF after traumatic injury with hypoperfusion on the day of injury, hyperemia on days 1–3 after injury, and vasospasm between days 4 and 15 after injury.

The late response to brain injury has been described primarily in human head injury and is characterized by return of CBF to normal or even elevated levels. Cerebral metabolic rate tends to remain reduced unless the patients rapidly recover consciousness and the arteriovenous difference of oxygen is low. Under the circumstances, even a normal level of CBF can be considered to be excessive for the reduced cerebral metabolic requirements (Obrist et al. 1984). Some investigators have observed a relationship between an elevated CBF and the development of intracranial hypertension (Kelly et al. 1996; Obrist et al. 1984).

**Immediate (first 5 min) NO response to injury**

The initial increase in NO concentration was most clearly defined using the NO electrode technique. There was not a consistent change in the dialysate concentrations of nitrate/nitrite during the first 20 min after the impact injury, although some animals did have an increase in the nitrite/nitrate concentration. The explanation for the differences in the two techniques during this time period is likely related to the transient nature of the increase in NO. The microdialysis technique does not have good time resolution. The samples were collected at 20-min intervals, and events lasting only a few minutes would likely be overlooked. A shorter sampling interval might improve the time resolution. Sakamoto et al. (1997) were able to demonstrate a nearly 2.5-fold increase in the concentration of NO metabolites in 10-min collections of dialysate after weight-drop injury. Nevertheless, the NO electrode is far superior for measuring transient changes in NO.

The mechanism of the initial increase in NO is not com-

**TABLE 2. P values for the repeated measures analysis for Figure 4**

<table>
<thead>
<tr>
<th></th>
<th>Group Effect</th>
<th>Time Effect</th>
<th>Group × Time Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracranial pressure</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>0.434</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dialysate nitrite/nitrate</td>
<td>0.071</td>
<td>&lt;0.001</td>
<td>0.008</td>
</tr>
</tbody>
</table>
pletely understood. Extraordinary metabolic and hemodynamic changes occur in the brain during this time period. At the time of a traumatic brain injury, widespread depolarization occurs. Potassium concentrations in the extracellular space dramatically increase and calcium accumulates intracellularly (Katayama et al. 1990, 1995; Kawamata et al. 1992). The brain has markedly increased glucose metabolic rates as the injured tissues reestablish normal ionic gradients (Hovda et al. 1990; Kawamata et al. 1992). Glutamate release may mediate some of these findings as kynurenic acid attenuates the injury-induced increased glucose metabolic rate (Hovda et al. 1990; Kawamata et al. 1992). A reasonable hypothesis for the increased NO based on these observations is that glutamate stimulation of NMDA receptors causes postsynaptic calcium influx and therefore activation of the constitutive isoforms of NOS.

In addition to the microdialysis study by Sakamoto (Sakamoto et al. 1997), others have observed an increase in NO or NOS activity immediately after traumatic brain injury using other techniques. Wada et al. (1998a) measured constitutive NOS (cNOS) activity 5 min and 30 min after lateral fluid percussion injury. An increase in cNOS activity to 243% of normal was observed at 5 min, with a return to baseline activity by 30 min after injury.

In one study using the fluid percussion injury model, administration of L-NAME 5 min before the injury markedly prolonged the systemic hypertension in response to the injury from 1 to 60 min (Lu et al. 1997). In addition, mortality rate was increased to 70% with preinjury administration of L-NAME. Death was primarily a result of pulmonary edema. This suggests that the systemic response to resolve the hypertension induced by the brain injury may involve NO production. However, in another study using the lateral fluid percussion injury model, administration of L-NAME both pre- and postinjury resulted in a significant increase in blood pressure; however, no adverse effect on outcome was observed (Wada et al. 1998a).

The brain tissue concentration of NO was consistently reduced between 5 min and 3 h after cortical impact injury using both techniques of measurement. The mechanism of the decrease in NO after traumatic brain injury is not well understood. The activity of cNOS has been measured after fluid percussion injury and follows the same general trend as the NO concentrations observed in this study (Wada et al. 1999). At 5 min, cNOS activity is increased, by 30 min cNOS activity is returned back to normal, and from 1 to 7 days after injury cNOS activity is reduced. A similar trend has been observed after middle cerebral artery occlusion, where at 10 and 20 min after occlusion cNOS activity is increased, by 1 h cNOS activity is returned to normal, and after day 1 cNOS activity is reduced (Iadecola et al. 1995; Kader et al. 1993).

Although CBF was not measured in this study, some deductions about the functional importance of these changes in NO may be drawn from past studies characterizing the CBF response to this injury model. When CBF has been measured using laser Doppler after the 5 m/s, 3-mm deformation impact injury, there was a small, very transient initial rise in CBF, followed by a 50% reduction in CBF that persists for at least 8 h (Cherian et al. 1994). In this respect, the CBF pattern follows that of NO observed in this study. In addition, when L-arginine is given 5 min after the impact injury, the reduced CBF is restored to near normal levels (Cherian et al. 1999).

Local depletion of substrate for NOS may play a role in reducing NO production (Leone et al. 1991; Rengasamy and Johns 1991). NO production during ischemia is initially increased. As oxygen supplies become limited, less citrulline is converted to arginine because that is an energy-dependent reaction. L-arginine administration increases NO production in this circumstance (Huk et al. 1997). This mechanism could partially explain decreased NO concentrations after traumatic brain injury because hypoperfusion and depletion of tissue oxygen occurs at the contusion site. L-arginine, possibly by increasing substrate availability, has been found to restore CBF in the cortical contusion and other models of traumatic brain injury (Cherian et al. 1999; DeWitt et al. 1997). The increased concentrations of citrulline observed in the dialysate of this experiment support impaired recycling of citrulline back to arginine, although the dialysate concentrations of arginine were not decreased.

### Table 3. P values for the repeated measures analysis for Figure 5

<table>
<thead>
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<th>Group Effect</th>
<th>Time Effect</th>
<th>Group × Time Interaction</th>
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<tr>
<td>Intracranial pressure</td>
<td>0.462</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>0.132</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
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### Early (5 min-3 h) NO response to injury

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Inhibition of NOS may also occur after trauma. Polyamines are known to be elevated after traumatic brain injury (Henley et al. 1996, 1997; Shohami et al. 1992). Cortical impact injury induced a 10- to 20-fold increase in ornithine decarboxylase (ODC) activity and a 4- to 5-fold increase in putrescine in the ipsilateral cortex. The adverse effects of polyamines in stroke and trauma are usually thought to be cytotoxic. Sparapini et al. (1997) found that polyamines (spermine > spermidine > putrescine) are toxic to granule cells in culture and that this toxicity is mediated through the NMDA receptor by interaction of exogenously added polyamines with endogenous glutamate released by neurons in the medium. However, polyamines may also have vascular effects by inhibiting NOS (Hu et al. 1994) or by blocking inward rectifier K⁺ channels (Ficker et al. 1994).

NO may be inactivated by free radicals, such as superoxide (Rubanyi and Vanhoutte 1986). Because free radicals are produced after traumatic brain injury (Kontos and Wei 1986), inactivation of NO by free radicals is another possible mechanism for the reduction in CBF. DeWitt (DeWitt et al. 1997) has shown that l-arginine infusion prevents the reduction in CBF and infusion of a free radical scavenger increases recovery of CBF after fluid percussion injury.

Late (>3 h after injury) NO response to injury

NO concentrations in the brain were not measured beyond 3 h postinjury in this study. Others, however, have examined NOS activity several days after injury. Whereas cNOS activity remains low for up to 7 days after injury, iNOS activity is increased on days 3 and 7 after injury (Wada et al. 1998b). This late NO production may have adverse effects on the injured brain.

Technical issues

Several other technical issues should be considered in assessing the early NO data of this study. The NO electrode must be removed during the impact injury and replaced for measurement because the measurements were obtained at the impact site. An initial increase in NO could be an artifact caused by the response of the brain tissue to puncture by the NO electrode. However, the sham injured animals did not experience an increased NO with replacement of the electrode, suggesting that the increase in NO observed in the injured animals was caused by the impact injury. Nevertheless an exacerbation of the injury-induced release of NO by the act of replacing the NO electrode cannot be ruled out. In addition, because the NO concentration was rapidly decreasing at the time of the NO electrode replacement, the peak NO concentration measured was probably considerably less than the actual peak NO concentration that occurred after the impact. The process of quickly and nontraumatically replacing the NO electrode after the trauma practically limited the NO measurements to one site. It is likely that the NO response varies with the location of the probe and the findings in this study which were obtained at the impact site cannot be generalized to other less severely injured areas of the brain.

Secondly, the NO electrode measures NO directly, whereas the dialysate nitrate/nitrite concentrations reflect oxidation of NO to NO₂ and NO₃ in the tissues. However, dialysate measurements of NO₂ and NO₃ have been shown to increase with administration of l-arginine, glutamate, and KCl, and these increases in NO₂ and NO₃ are blocked by l-NAME administration (Ohta et al. 1994; Yamada and Nabeshima 1997). All of these findings suggest that the dialysate concentrations of NO₂ and NO₃ reflect NO activity.

Summary

Immediately after impact injury NO is markedly increased. Within minutes, NO concentration falls and remains an average of 25 nM lower than baseline values for at least 3 h after injury, which was the duration of monitoring in this study. Both techniques for measuring NO production gave similar results except that the immediate transient increase in NO was best demonstrated with the NO electrode. These data suggest that reduced NO production may play a role in the reduction of CBF after severe traumatic brain injury.

Address for reprint requests: L. Cherian, Dept. of Neurosurgery, Baylor College of Medicine, 6560 Fannin St., Suite 944, Houston, TX 77030.

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