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Early and sharp nitric oxide production and anoxic depolarization in the rat hippocampus during transient forebrain ischemia

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Abstract

This study was designed to characterize nitric oxide (NO) production and anoxic depolarization in the rat hippocampus during transient forebrain ischemia using two NO synthase (NOS) inhibitors, L-N^5-(1-iminoethyl)ornithine (L-NIO), a relatively selective endothelial NOS (eNOS) inhibitor, and 7-nitroindazole, a relatively selective neuronal NOS (nNOS) inhibitor, and an NO scavenger, [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide] (carboxy-PTIO). We measured the mean arterial blood pressure, hippocampal blood flow, NO concentration and direct current potential before, during and after transient forebrain ischemia, which was induced by 4-vessel occlusion for 10 min. Saline, L-NIO (20 mg/kg), 7-nitroindazole (25 mg/kg), L-NIO (20 mg/kg)+7-nitroindazole (25 mg/kg) or carboxy-PTIO (1 mg/kg) was administered intraperitoneally 20 min before the onset of ischemia. We observed early and sharp NO production in the hippocampus during ischemia in the saline group. This NO increase during ischemia was significantly reduced by L-NIO (20 mg/kg)+7-nitroindazole (25 mg/kg) or carboxy-PTIO (1 mg/kg), but not L-NIO (20 mg/kg) or 7-nitroindazole (25 mg/kg). On the other hand, NO production after ischemia was significantly reduced by 7-nitroindazole (25 mg/kg), L-NIO (20 mg/kg)+7-nitroindazole (25 mg/kg) or carboxy-PTIO (1 mg/kg), but not L-NIO (20 mg/kg). The peak latency of NO production during ischemia always preceded the onset latency of anoxic depolarization in both the saline group and the carboxy-PTIO group. In the carboxy-PTIO group, the onset latency of anoxic depolarization was significantly longer than that in the saline group. Moreover, carboxy-PTIO significantly reduced the anoxic depolarization amplitude, compared with that of the saline group. These results suggest that both NOS-dependent and-independent NO formation contributes to early and sharp NO production during ischemia, and that this NO increase is, at least in part, related to the triggering of anoxic depolarization.

Keywords: Anoxic depolarization; Cerebral ischemia; Nitric oxide; Nitric oxide synthase inhibitor; Nitric oxide scavenger

1. Introduction

Previous studies have demonstrated an increased production of nitric oxide (NO) in the brain during ischemia (Malinski et al., 1993; Huang et al., 1994; Ohta et al., 1997; Jiang et al., 1999). The available evidence suggests that NO is generated by both neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS) at the beginning of ischemia (Huang et al., 1994; Grandati et al., 1997; Ohta et al., 1997; Jiang et al., 1999, 2002). In our previous study (Jiang et al., 1999), we demonstrated that the increased NO production from the rat hippocampus during post-ischemic early reperfusion is dependent on nNOS. Although we also observed an early and sharp increase in NO production, which occurs at the early stage of ischemia, we did not analyze the origins of NO. Previous studies (Malinski et al., 1993; Ohta et al., 1996, 1997) did not describe an early and sharp NO increase during focal cerebral ischemia.

The NO concentration increased in severely ischemic regions exhibiting anoxic depolarization in the cat model of focal cerebral ischemia (Ohta et al., 1996). This occurred without a reduction in extracellular Ca^{2+} concentration before a massive Ca^{2+} influx into cells several minutes later. NO produced Ca^{2+}-independent glutamate release from rat synaptosomes (McNaught and Brown, 1998). Higher increases of extracellular glutamate were observed when the ischemic insult produced anoxic depolarization (Ueda et al., 1992). We showed that a relatively selective

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Table 1
Effects of L-NIO, 7-nitroindazole (7-NI), L-NIO+7-NI and carboxy-PTIO on changes in hippocampal blood flow induced by 10 min of forebrain ischemia

Group	Value at 20 min after drug administration (%)	Minimal value during ischemia (%)	Hyperemia value after ischemia (%)	Value at 60 min after reperfusion (%)
Saline (n=8)	106.3±1.7	11.4±0.9	261.6±13.6	75.7±6.5
L-NIO (n=4)	101.7±3.7	7.7±0.5	219.5±28.7	79.7±11.7
7-NI (n=4)	101.8±0.6	8.8±0.5	270.8±34.0	94.5±5.0
L-NIO+7-NI (n=4)	94.1±1.4	3.3±0.7	257.0±37.3	56.8±7.1
Carboxy-PTIO (n=5)	103.4±2.5	2.6±0.9	244.6±13.4	86.1±3.7

Values are the mean±S.E.M. During ischemia, hippocampal blood flow showed the lowest value in any group. One-way ANOVA with repeated measures revealed a significant main effect of time [$F(3, 60)=332.659$, $P<0.0001$], but there were no significant differences of the main effect of group [$F(4, 20)=1.106$, $P<0.3810$] and group×time [$F(12, 60)=0.815$, $P<0.6340$].

nNOS inhibitor, 7-nitroindazole, reduced the amplitude of anoxic depolarization induced by ischemia, although it did not affect the onset latency of anoxic depolarization (Jiang et al., 2002).

To the best of our knowledge, there are no available studies regarding the relationship between this NO increase and anoxic depolarization in a 4-vessel occlusion ischemia model. In the present study, we focused on the early and sharp increase in NO production during ischemia. Therefore, we attempted to clarify the origins of this NO production, and to characterize the temporal relationship between this NO increase and the occurrence of anoxic depolarization. We therefore simultaneously measured NO levels using an NO-selective electrode and direct current potential using a glass-microelectrode in the rat hippocampus before, during and after transient forebrain ischemia. The pharmacological tools used to inhibit eNOS and nNOS were a relatively selective eNOS inhibitor, L-N^5-(1-iminoethyl)ornithine (L-NIO) (Mulligan et al., 1992; Wolff et al., 1998; McDuffie et al., 2000, Rees et al., 1990), and 7-nitroindazole (Moore et al., 1993; Yoshida et al., 1994; Schulz et al., 1995; Jiang et al., 2002), respectively. Moreover, carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO) (Amano and Noda, 1995; Kagiyama et al., 1997; Gomez-Vargas et al., 1998) was used to scavenge NO.

2. Materials and methods

2.1. Animal preparation

Animal experiments were conducted with the approval of the Animal Care and Use Committee of Hyogo College of Medicine and according to The Guiding Principles for the Care and Use of Animals approved by the Council of The Physiological Society of Japan.

Male Wistar rats weighing between 310 and 360 g were used. The day before the experiment, the rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and the bilateral vertebral arteries were electrocauterized. The next day, the rats were anesthetized with urethane (1.2 g/kg, i.p.). The bilateral common carotid arteries were dissected and suture loops were placed around each vessel. A femoral artery was catheterized for continuous monitoring of mean arterial blood pressure (MABP). The rat was then placed in a stereotaxic frame. Rectal temperature was maintained at 37–38 °C with a heating pad. A temperature sensor was carefully placed on the brain surface, and the brain temperature was maintained at 36–37 °C with a lamp. After each experiment, the PO_2, PCO_2 and pH of the arterial blood were analyzed with a portable clinical analyzer (i-STAT 200A, i-STAT, NJ, USA).

2.2. Measurement of hippocampal blood flow

A laser Doppler flowmetry probe (Needle Type ON97-066, tip diameter 500 μm, Unique Medical, Tokyo, Japan) was stereotaxically inserted into the left dorsal hippocampus (5.5 mm caudal to the bregma, 4.0 mm lateral to the midline and 3.0 mm below the cortical surface). Hippocampal blood flow was measured with laser Doppler flowmetry (TBF-LC1, Unique Medical, Tokyo, Japan). Hippocampal blood flow data were expressed as a percentage of the baseline value before drug administration.

2.3. Measurement of hippocampal direct current potential

The electrode recording direct current potentials consisted of a glass-micropipette with a tip diameter of about 10–20 μm, which was filled with 2% pontamine sky blue in 0.5 M sodium acetate. This electrode was inserted into the stratum pyramidale of the CA1 in the right dorsal hippocampus (5.5 mm caudal to the bregma, 3.0 mm lateral to the midline and 3.0 mm below the cortical surface). The direct current potential was monitored with a direct current amplifier (Nihon Kohden, Tokyo, Japan). The onset latency of anoxic depolarization was measured from the start of ischemia to sudden depolarization. The amplitude of anoxic depolarization was measured from the first positive peak to the first negative peak.

Table 2
Effects of L-NIO, 7-nitroindazole (7-NI), L-NIO+7-NI and carboxy-PTIO on changes in mean arterial blood pressure (MABP) induced by 10 min of forebrain ischemia

Group	Baseline MABP (mmHg)	MABP at 20 min after drug administration (mmHg)	Maximal MABP during ischemia (mmHg)	MABP at 60 min after reperfusion (mmHg)
Saline (n=8)	74.8±1.6	75.4±2.0	155.4±7.1	86.4±3.5
L-NIO (n=4)	74.8±1.5	85.3±2.1	221.5±17.7	132.3±8.9
7-NI (n=4)	81.5±1.3	82.5±1.3	212.3±3.2	99.5±3.6
L-NIO+7-NI[a] (n=4)	75.3±2.1	76.8±6.8	271.8±7.0	138.8±6.0
Carboxy-PTIO (n=5)	78.0±1.5	86.4±2.0	223.4±8.1	106.4±1.8

Values are the mean±S.E.M. After the onset of ischemia, MABP showed the maximal values in any group. One-way ANOVA with repeated measures revealed a significant main effect of time [$F(4, 20)=24.727$, $P<0.0001$], time [$F(3, 60)=804.933$, $P<0.0001$] and a significant difference in group×time [$F(12, 60)=18.859$, $P<0.0001$]. [a]$P<0.0183$, compared with the saline group (post-hoc Fisher's PLSD test).