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Maternal treatment with NMDA receptor antagonist (MK-801) attenuates delayed mitochondrial dysfunction after transient intrauterine ischemia in the neonatal rat brain

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Abstract

Purpose. Mitochondrial respiratory activities were measured in neonatal rat brains to evaluate the influence of transient intrauterine ischemia on the near-term fetus and to assess the effect of dizocilpine maleate (MK-801), a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist.
Methods. Transient intrauterine ischemia was induced by 30 min of right uterine artery occlusion at 17 days of gestation in Wistar rats. Vehicle (saline) or 0.5 mg/kg of MK-801 was administered after 1 h of recirculation. All of the pups were delivered by cesarean section at 21 days of gestation and samples of cerebral cortical tissue were obtained from pups at 1 h after birth. Adenosine diphosphate (ADP)-stimulated, nonstimulated, and uncoupled respirations were measured polarographically in homogenates. The respiratory control ratio was defined as ADP-stimulated divided by nonstimulated respiration.
Results. In the vehicle-treated group the neonatal cortical tissue exposed to ischemia showed a significant decrease in ADP-stimulated respiration and respiratory control ratio compared with these findings in normoxic control animals. The delayed mitochondrial respiratory dysfunction was prevented by MK-801, given 1 h after the start of recirculation ($P < 0.05$).
Conclusion. The present results indicate that transient intrauterine ischemia in the near-term rat fetus is associated with delayed mitochondrial dysfunction in the neonatal brain; the results suggest that maternal treatment with MK-801 attenuates the deterioration, even when administered 1 h after the start of recirculation.

Key words Intrauterine ischemia · Neonate · Brain · Mitochondria · MK-801

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Introduction

It has been established that excitatory amino acids, notably glutamate, play a crucial role in ischemic neuronal death [1]. The N-methyl-D-aspartate (NMDA) receptor, an ionotropic glutamate receptor found in the central nervous system, has several well-characterized regulatory and functional binding sites, including the glutamate (recognition) site, the glycin (co-activator) site, and a site within the receptor-associated ion channel that binds phencyclidine and the noncompetitive antagonist dizocilpine maleate (MK-801) [2]. Several studies have demonstrated that treatment with MK-801 after hypoxia-ischemia reduces subsequent morphologic brain damage in adult [3] and neonatal rats [4]. This drug also decreases the size of areas with an increased cerebral metabolic rate of glucose when given 1 to 5 h after hypoxia-ischemia [5]. In newborn humans with hypoxic-ischemic encephalopathy, regional increases in the cerebral metabolic rate of glucose predict the later appearance of neurologic deficits that correspond to damage in these brain areas [6]. NMDA receptor activation after hypoxia-ischemia may contribute to the development of infarction by affecting energy metabolism. The mechanisms for this are unknown, and may reflect the finding that NMDA receptor activation enhances the energy requirements of tissue or exacerbates mitochondrial function during reperfusion.

Maternal uterine artery ligation has frequently been used to study the consequences of reduced uterine blood flow on the fetus. This procedure has been shown to produce fetal hypoxia, hypercapnia, and acidosis [7–10], which likely affect fetal cellular metabolism. Indeed, in our preliminary studies, we reported that recirculation after transient maternal uterine artery occlusion was associated with an initial, partial recovery of the cellular bioenergetic state and of mitochondrial function, with secondary deterioration occurring during the first 2–4 h of reflow, in the term fetal rat brain

[11,12]. In addition, 30 min of uterine artery occlusion in pregnant rats at 17 days of gestation was found to cause an asymmetric retardation of fetal growth [10]. In the present study, we used the same experimental approach to detect the changes in mitochondrial respiratory activities in neonatal rat brains and to assess the effect of MK-801 treatment. The hypothesis tested was whether enhanced activation of NMDA receptors is implicated in the development of neonatal neurologic deficit, seen as delayed mitochondrial dysfunction.

Materials and methods

This study was approved by the Ethics Committee for Animal Experimentation at our university. Animal care complied with the "Guidelines for Care of Experimental Animals" issued by the Office of the Prime Minister of Japan.

Drug administration and experimental protocol

MK-801, purchased from Sigma Chemical (St. Louis, MO, USA), was diluted ten times with saline for intravenous use. MK-801 was injected at a dose of $0.5\,mg \cdot kg^{-1}$ into the maternal tail vein. Studies were carried out in the following three groups of pregnant rats: (a) sham-operated group ($n = 6$ pregnant rats), in which procedures were identical to those in the experimental groups except that uterine artery occlusion was not induced; (b) ischemia group ($n = 6$ pregnant rats), in which transient intrauterine ischemia was produced by 30-min uterine artery occlusion and vehicle (saline) injections were given; and (c) ischemia with MK-801 group ($n = 6$ pregnant rats), in which transient intrauterine ischemia was produced and MK-801 injections were given. MK-801 or vehicle (saline) was administered intravenously to pregnant rats 1 h after the start of recirculation.

Animal preparation and transient intrauterine ischemia

Ten-week-old pregnant Wistar rats (Sankyo Lab Service, Tokyo, Japan), weighing 250–300 g, were used. The rats were housed separately at ambient temperature with a 12-h light cycle, and were allowed free access to water and food. At 17 days of gestation (term, 21.5 days), the animals were anesthetized with 3% halothane in a mixture of $N_2O:O_2$ (70:30) after overnight fasting. They were then intubated and artificially ventilated on 1.0% to 1.5% halothane during the operation. The maternal tail artery was cannulated to measure arterial blood gases, arterial pH, blood glucose, and blood pressure. The maternal tail vein was also cannulated for MK-801 or saline intravenous injections. A midline abdominal incision was performed, and the two uterine horns were exposed and kept moist with saline. Transient uterine artery occlusion was induced according to the technique of Ishimoto et al. [10]. Briefly, two microvascular clips were used to occlude the uterine vessels near the lower and upper ends of the right uterine horn. The clips were removed after 30 min of ischemia. During the operation a core temperature of 37.0°C was regularly maintained with a heating pad. For each experiment, the fetuses in the right uterine horn served as the ischemia group and those in the left horn as the non-ischemia group. At 21 days of gestation the animals were re-anesthetized, tracheotomized, and artificially ventilated. After the physiological parameters had been stabilized for at least 5 min, the fetuses were delivered by cesarean section and weighed to determine the degree of intrauterine fetal growth retardation. To keep sampling conditions stable, only one fetus was sampled from the middle portion of each uterine horn for the measurement of mitochondrial respiratory function. Other live fetuses were weighed to determine the degree of intrauterine fetal growth retardation. The fetal brains and livers were also weighed.

Measurement of mitochondrial respiratory function

All pups were decapitated at 1 h after delivery. The whole brains were removed rapidly, within 60 s, and transferred to an ice-cold homogenization buffer containing sucrose, 0.32 mol/l, potassium-ethylenediaminetetraacetic acid, 1 mmol/l, and tris (hydroxymethyl) aminomethane (Trizma Base; Sigma), 10 mmol/l, that had been adjusted to pH 7.4 with a pH monitor (Inter Medical, Tokyo, Japan). The forebrain was dissected out and washed free of blood with an ice-cold homogenization buffer, and the surface cleared of meninges and vessels. The brains yielded 100–150 mg of wet tissue.

Tissue samples were homogenized in homogenization buffer using an all-glass Dounce homogenizer, according to a technique described by Sims and Blass [13]. We used these homogenates to measure mitochondrial respiration. This technique measures the function of the whole population of free brain mitochondria [13–15].

Mitochondrial respiration in prepared homogenates was measured polarographically with an oxygen microelectrode (Presearch, Hertfordshire, United Kingdom) in a closed, magnetically stirred chamber with a capacity of 770 μl, at 28°C (YSI, Hampshire, United Kingdom). Samples (usually 80–100 μl) were added to the reaction buffer (usually 0.65–0.7 ml) containing potassium chloride, 100 mmol/l; mannitol, 75 mmol/l; sucrose, 25 mmol/l; tris-phosphate, 5 mmol/l; potassium-ethylenediaminetetraacetic acid, 0.05 mmol/l; and tris (hydroxymethyl) aminomethane (Trizma

Base), 10 mmol/l (pH 7.4). Substrates consisting of 10 μl of glutamate (0.5 mol/l) and malate (0.5 mol/l), neutralized with potassium hydroxide, were also added. Respiration stimulated with adenosine diphosphate (ADP) was initiated by the addition of ADP, 0.1 mol/l (typically, 0.3 μl followed by 5 μl). Respiration not stimulated with ADP was measured from tracings obtained after depletion of the ADP that had been added to stimulate respiration and after decline in the rate to a constant value. Uncoupled respiration was initiated by the addition of 5 μl carbonyl cyanide *m*-chlorophenylhydrazone, 0.9 mmol/l [12–18]. The respiratory control ratio (RCR), which reflects the coupling of respiration to ATP synthesis, was calculated as the ratio of stimulated to nonstimulated respiration. At the conclusion of measurement, an aliquot (0.2 ml) was removed and frozen (−18°C) for subsequent measurement of protein content, carried out with a DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA) [19].

Statistical analysis

All data values were expressed as means ± SDs. One-way analysis of variance (ANOVA) followed by Scheffé's *F*-test was used to compare the values within each experimental group. The Mann-Whitney *U*-test was used to compare the values between ischemia and non-ischemia uterine horns. Differences with a *P* value of less than 0.05 were considered to be statistically significant.

Results

The physiological parameters of maternal animals during 30-minute uterine artery occlusion are shown in Table 1. There were no significant differences in blood pressure, arterial PO_2, PCO_2, pH, and glucose concentrations during operation between the experimental groups. The rats given MK-801 showed no significant difference from vehicle-treated rats in core temperature during and following uterine artery occlusion.

The neonatal body weights and weights of organs and placenta are shown in Table 2. In the non-ischemic uterine horn, there were no significant differences in the weights of neonatal body, organs, and placenta among the three experimental groups. In the ischemic uterine horn, the weights of neonatal body, placenta, and liver of the vehicle-treated animals were significantly lower than those in the non-ischemic uterine horn. In contrast to the results obtained from the vehicle-treated animals, there were no significant differences in the weights of the neonatal body, organs and placenta of MK-801 treated animals between the ischemic and the non-ischemic uterine horns.

ADP-stimulated, nonstimulated, and uncoupled O_2 consumption rates were calculated from polarographic tracings. The mitochondrial respiratory activities for all groups are shown in Table 3. In the non-ischemic uterine horn, there were no significant differences in ADP-stimulated, non-stimulated, and uncoupled respiration among the three experimental groups. In the ischemic uterine horn, the ADP-stimulated respiration of the ischemia group with vehicle treatment decreased significantly to about 65% of that in the non-ischemic controls, but non-stimulated respiration remained unchanged. Therefore, RCR decreased significantly to about 57% of that in the non-ischemic controls. Uncoupled respiration in the ischemia group with vehicle treatment showed changes that were similar to those in ADP-stimulated respiration, but these differences from non-ischemic contrals were not significant.

Table 1. Physiological parameters of the pregnant rats used in this study

	Sham-operated (n = 6)	Ischemia with vehicle (n = 6)	Ischemia with MK-801 (n = 6)
Blood pressure (mmHg)	110.2 ± 10.1	105.7 ± 12.1	108.8 ± 12.5
P_{O2} (mmHg)	145.3 ± 11.5	140.8 ± 16.8	149.0 ± 15.8
P_{CO2} (mmHg)	39.6 ± 2.9	40.5 ± 3.0	40.2 ± 2.0
pH	7.42 ± 0.03	7.39 ± 0.03	7.39 ± 0.05
Blood glucose (mg/dl)	79.4 ± 10.1	80.8 ± 12.9	75.8 ± 15.1
Core temperature (°C)			
0-min occlusion	36.7 ± 0.4	36.5 ± 0.6	36.7 ± 0.3
30-min occlusion	37.1 ± 0.4	36.9 ± 0.4	37.0 ± 0.5
1-h recirculation	37.1 ± 0.5	37.0 ± 0.2	37.1 ± 0.3
1-day recirculation	36.9 ± 0.3	36.8 ± 0.3	36.9 ± 0.3
3-day recirculation	36.8 ± 0.4	36.8 ± 0.3	36.7 ± 0.4

Values are means ± SDs.
There were no significant differences between the three groups for any of these physiological parameters.

Table 2. Neonatal body weight and organ weights

	Sham-operated	Ischemia with vehicle	Ischemia with MK-801
Non-ischemic uterine horn	(n = 25)	(n = 29)	(n = 25)
Neonatal body weight (mg)	3179 ± 311	3269 ± 290	3344 ± 318
Brain weight (mg)	145 ± 15	158 ± 18	155 ± 21
Liver weight (mg)	122 ± 21	120 ± 14	133 ± 29
Placenta weight (mg)	577 ± 72	551 ± 50	581 ± 85
Ischemic uterine horn	(n = 24)	(n = 23)	(n = 27)
Neonatal body weight (mg)	3301 ± 321	2661 ± 254*	3117 ± 307**
Brain weight (mg)	151 ± 12	143 ± 20	146 ± 18
Liver weight (mg)	130 ± 26	89 ± 27*	118 ± 25**
Placenta weight (mg)	524 ± 89	497 ± 69*	513 ± 81

* Versus non-ischemic uterine horn; Mann-Whitney U-test ($p < 0.05$); ** Versus ischemia with vehicle, one-factor analyis of variance (ANOVA) followed by Scheffe's F-test ($P < 0.05$).
Values are means ± SDs.

Table 3. ADP-stimulated, non-stimulated, and uncoupled respiration (nmol/O_2 per min per mg protein) and RCR in homogenized brain tissue

	Sham-operated (n = 6)	Ischemia with vehicle (n = 6)	Ischemia with MK-801 (n = 6)
Non-ischemic uterine horn			
ADP-stimulated respiration	19.4 ± 4.3	21.2 ± 3.3	19.6 ± 4.4
Non-stimulated respiration	4.8 ± 1.1	5.0 ± 0.7	4.9 ± 1.2
Uncoupled respiration	26.6 ± 4.1	26.2 ± 3.6	25.5 ± 6.5
RCR	4.0 ± 0.3	4.2 ± 0.4	4.1 ± 0.3
Ischemic uterine horn			
ADP-stimulated respiration	20.1 ± 4.5	12.8 ± 3.2*	18.7 ± 2.5**
Non-stimulated respiration	4.5 ± 0.9	5.2 ± 1.2	5.6 ± 1.5
Uncoupled respiration	25.9 ± 5.0	19.9 ± 4.5	23.3 ± 7.0
RCR	4.4 ± 0.5	2.4 ± 0.4*	3.5 ± 0.5**

* Versus non-ischemic uterine horn, Mann-Whitney U-test ($P < 0.05$). ** Versus ischemia with vehicle, one-factor ANOVA followed by Scheffe's F-test ($P < 0.05$).
Values are means ± SDs.
ADP, Adenosine diphosphate; RCR, respiratory control ratio.

Treatment with MK-801 in the ischemic uterine horn group caused normalization of ADP-stimulated and uncoupled respiration, to about 95% and 90% of the levels in non-ischemic controls, respectively. MK-801 also significantly increased RCR, compared with that in the ischemia group with vehicle treatment (Table 3).

Discussion

The present study demonstrated that treatment with MK-801 ameliorated both the growth retardation and the cerebral mitochondrial dysfunction in neonatal rats after intrauterine ischemia produced by transient uterine artery occlusion. Although there is no evidence of neuronal necrosis or apoptosis that occur in this model, the results suggest that enhanced activation of NMDA receptors is implicated in the development of neonatal neurologic deficit.

As reported recently [10,17,20], transient uterine artery occlusion in pregnant rats causes an asymmetric retardation of fetal growth. In agreement with these reports, our results on the growth retardation in vehicle-treated animals demonstrated significant differences in the weights of the neonatal body, organs, and placenta between the non-ischemic and ischemic uterine horns. In contrast to the findings in the vehicle-treated animals, MK-801 exerted a protective effect on the growth retardation.

Our preliminary studies, in which we used the same experimental approach as we used in this study, showed that recirculation after transient intrauterine ischemia was associated with an initial, partial recovery of the cellular bioenergetic state and of mitochondrial function, with secondary deterioration during the first 2–4 h of reflow in the term fetal rat brain [11,12]. The results are, thus, similar to those obtained in mature animals subjected to transient middle cerebral artery occlusion

[14,15,21]. In the present study, the results obtained in the vehicle-treated animals suggest that the secondary mitochondrial dysfunction was still present 1h after birth (4 days after recirculation). The mitochondrial dysfunction that develops in the recirculation period encompasses not only a decrease in ADP-stimulated respiratory activities but also an increase in mitochondrial membrane permeability to large molecules. It has now been convincingly demonstrated that a decrease in the membrane potential, leading to a mitochondrial permeability transition (MPT), is a trigger of apoptotic cell death, and possibly, also of necrotic cell death [22,23]. It is envisaged that a decrease in the membrane potential of sufficient magnitude to elicit an MPT leads to the production of reactive oxygen species and the release of apoptogenic factors, such as cytochrome c. Thus, the present results obtained in the vehicle-treated animals suggest that the neurologic deficit apparent in neonates may be attributed, in part, to events traditionally considered as apoptotic.

MK-801, a potent noncompetitive antagonist of the NMDA glutamatergic receptor subtype, can readily cross the blood-brain barrier [24]. Previous studies in adult animals have demonstrated that MK-801 reduced brain injury after focal ischemia [3,25], and these authors suggested that one important component of neuroprotection with this drug was the reduction of glutamate efflux, leading to a decrease in excitotoxic injury. Elevated excitotoxins can lead to an increase in intracellular and mitochondrial free calcium [26]. Recent studies demonstrate that mitochondria are a primary target of glutamate toxicity [27]. Glutamate-mediated massive influx of calcium in mitochondria triggers a decrease in the membrane potential and the opening of an MPT pore, which may contribute to cell death [14,15,28]. Indeed, Gilland et al. [29] demonstrated that treatment with MK-801 improved mitochondrial function in the 7-day-old rat brain after hypoxia-ischemia. However, whether a similar mechanism for the protective effect of this drug plays a role in the fetal brain remains an unresolved issue. Gordon et al. [30] found only a variable threefold elevation of extracellular glutamate and aspartate during hypoxia-ischemia in the immature rat compared with the greater than 60-fold increase generally seen in adult brain undergoing ischemic stress. Cherici et al. [31] also reported that ischemia did not induce the release of excitotoxic amino acids from the hippocampus of the newborn rat. However, Kristian and Siesjö [32] suggested that even a minor increase in the release of glutamate may be a maximal trigger of calcium influx into postsynaptic elements. Based on these results, we suggest that the protective effect of MK-801 on neonatal mitochondrial activities may be caused, in part, by the decreased influx of calcium in mitochondria.

Another possible explanation for the beneficial effect is that MK-801 improved the perfusion of the whole fetoplacental unit after 30min of ischemia. However, as far as we know, there are no comparable studies that have demonstrated the effect of MK-801 on fetoplacental perfusion during the recirculation period after transient intrauterine ischemia.

These data are consistent with the hypothesis that enhanced activation of NMDA receptors is implicated in the secondary phase of mitochondrial respiratory dysfunction, which may lead to the development of a neonatal neurologic deficit. Maternal hypertension, chronic renovascular disease, and several other conditions that are associated with reduced uterine blood flow during pregnancy, appear to be important pathophysiologic events in the mechanism of injury to the immature fetal brain [33–35]. Indeed, an increasing number of clinical reports suggests that much of the neurologic deficit apparent in neonates can be attributed to prenatal hypoxic-ischemic events [36,37]. We believe that the results of this study have the potential to provide new insights into the mechanisms of these disorders; we suggest the need for further studies of this field.

In conclusion, the present results indicate that transient intrauterine ischemia in the near-term rat fetus is associated with delayed mitochondrial dysfunction in the neonatal brain; the results suggest that maternal treatment with MK-801 attenuates the deterioration, even when administered 1h after the start of recirculation.

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References

1. Siesjö BK, Zhao Q, Pahlmark K, Siesjo P, Katsura K, Folbergrova J (1995) Glutamate, calcium, and free radicals as mediators of ischemic brain damage. Ann Thorac Surg 59:1316–1320
2. Wood PL, Rao TS, Iyengar S, Lanthorn T, Monahan J, Corki A, Sun E, Vazquez M, Gray N, Contreras P (1990) A review of the in vitro and in vivo neurochemical characterization of the NMDA/PCP/glycine/ion channel receptor macrocomplex. Neurochem Res 15:217–230
3. Park CK, Nehls DG, Graham DI, Teasdale GM, McCulloch J (1988) The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. Ann Neurol 24:543–551
4. Hagberg H, Gilland E, Diemer NH, Andine P (1994) Hypoxia-ischemia in the neonatal rat brain: histopathology after post-treatment with NMDA and non NMDA receptor antagonists. Biol Neonate 66:206–213
5. Gilland E, Hagberg H (1996) NMDA-receptor dependent increase of cerebral glucose utilization after hypoxia-ischemia in the neonatal rat. J Cereb Blood Flow Metab 16:1005–1013
6. Blennow M, Ingvar M, Lagercrantz H, Stone ES, Eriksson L, Forssberg H, Ericson K, Flodmark O (1995) Early [18F]FDG

position emission tomography in infants with hypoxic-ischemic encephalopathy shows hypermetabolism during the postasphyctic period. Acta Paediatr 84:1289–1295
7. Wigglesworth JS (1964) Experimental growth retardation in the foetal rat. J Pathol Bacteriol 88:1–13
8. Ogata ES, Bussey M, Finley S (1989) Altered gas exchange, limited glucose and branched chain amino acids, and hypoinsulinemia retard fetal growth in the rat. Metabolism 35:970–977
9. Yavin E, Inserte-Igual J, Gil S (1995) Ischemia-triggered translocation and inactivation of protein kinase C isoforms in the fetal brain. J Neurochem 65:2594–2602
10. Ishimoto H, Natori M, Tanaka M, Miyazaki T, Kobayashi T, Yoshimura Y (1997) Role of oxygen-derived free radicals in fetal growth retardation induced by ischemia-reperfusion in rats. Am J Physiol 272:701–705
11. Nakai A, Asakura H, Taniuchi Y, Koshino T, Araki T, Siesjö BK (2000) Effect of a-phenyl-N-tert-butyl nitrone (PBN) on fetal cerebral energy metabolism during intrauterine ischemia and reperfusion in rats. Pediatr Res 47:451–456
12. Nakai A, Taniuchi Y, Asakura H, Yokota A, Koshino T, Araki T (2000) Secondary mitochondrial dysfunction after transient intrauterine ischemia in the fetal brain. J Obstet Gynecol Res 26:383–387
13. Sims N, Blass J (1986) Expression of classical mitochondrial respiratory responses in homogenates of rat forebrain. J Neurochem 47:496–505
14. Kuroda S, Katsura K, Hillered L, Bates TE, Siesjö BK (1996) Delayed treatment with a-phenyl-N-tert-Butyl-nitrone (PBN) attenuates secondary mitochondrial dysfunction after transient focal ischemia in the rat. Neurobiol Dis 3:149–157
15. Nakai A, Kuroda S, Kristian T, Siesjö BK (1997) The immunosuppressant drug FK506 ameliorates secondary mitochondrial dysfunction folowing transient focal cerebral ischemia in the rat. Neurobiol Dis 4:288–300
16. Nakai A, Taniuchi Y, Asakura H, Oya A, Yokota A, Koshino T, Araki T (2000) Developmental changes in mitochondrial activity and energy metabolism in fetal and neonatal rat brain. Dev Brain Res 121:67–72
17. Shibazaki Y, Nakai A, Koshino T, Yokoyama K (2001) Effect of the immunosuppressant drug FK506 on neonatal cerebral mitochondrial function and energy metabolism after transient intrauterine ischemia in rats. Brain Res 892:351–358
18. Nakai A, Taniuchi Y, Asakura H, Oya A, Yokota A, Koshino T, Araki T (2001) Developmental changes in tolerance to transient intrauterine ischemia in rat cerebral mitochondria. Am J Obstet Gynecol 184:731–735
19. Lowry O, Rosebrough N, Farr A, Randall R (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275
20. Tanaka M, Natori M, Ishimoto H, Miyazaki T, Kobayashi T, Nozawa S (1994) Experimental growth retardation produced by transient period of uteroplacental ischemia in pregnant Sprague-Dawley rats. Am J Obstet Gynecol 171:1231–1234
21. Folbergrova J, Zhao Q, Katsura K, Siesjö BK (1995) N-tert-a-phenylnitrone improves recovery of brain energy state in rats following transient focal ischemia. Proc Natl Acad Sci USA 92:5057–5061
22. Zamzami N, Susin SA (1996) Mitochondrial control of nuclear apoptosis. J Exp Med 183:1533–1544
23. Hirsch T, Marzo I, Kroemer G (1997) Role of the mitochondrial permeability transition pore in apoptosis. Biosci Rep 17:67–76
24. Papagapiou MP, Auer RN (1990) Regional neuroprotective effects of the NMDA receptor antagonist MK-801 (dizoclipine) in hypoglycemic brain damage. J Cereb Blood Flow Metab 10:270–276
25. Margaill I, Parmentier J, Allix M, Boulu R, Plotkine M (1996) Short therapeutic window for MK-801 in transient focal cerebral ischemia in normotensive rats. J Cereb Blood Flow Metab 16:107–113
26. Kristian T, Gido G, Kuroda S, Schutz A, Siesjö BK (1998) Calcium metabolism of focal and penumbral tissue in the rats subjected to transient middle cerebral artery occlusion. Exp Brain Res 120:503–509
27. Schinder AF, Olson EC, Spitzer NC, Montal M (1996) Mitochondrial dysfunction is primary event in glutamate neurotoxicity. J Neurosci 16:6125–6133
28. Ouyang Y-B, Kuroda S, Kristian T, Siesjö BK (1997) Release of mitochondrial aspartate aminotransferase (mAST) following transient focal ischemia suggests the opening of a mitochondrial permeability transition pore. Neurosci Res Comm 20:167–173
29. Gilland E, Puka-Sundvall M, Hillered L, Hagberg H (1998) Mitochondrial function and energy metabolism after hypoxia-ischemia in the immature rat brain: involvement of NMDA-receptor. J Cereb Blood Flow Metab 18:297–304
30. Gordon KE, Simpson J, Statman D, Silverstein FS (1991) Effects of perinatal stroke on striatal amino acid efflux in rats studied with in vivo microdialysis. Stroke 22:928–932
31. Cherici G, Alesiani M, Pellegrini-Giampietro DE, Moroni F (1991) Ischemia does not induce the release of excitotoxic amino acids from hippocampus of newborn rats. Dev Brain Res 1991: 235–240
32. Kristian T, Siesjö BK (1992) The influence of moderate hypothermia on cellular calcium uptake in complete ischemia: implication for the excitotoxic hypothesis. Acta Physiol Scand 146:531–532
33. Ting P, Yamaguchi S, Bacher JD, Killens RH, Myers RE (1983) Hypoxic-ischemic cerebral necrosis in midgestational sheep fetuses: pathophysiologic correlation. Exp Neurol 80:227–245
34. Wagner RE, Ting P, Westfall MV, Yamaguchi S, Bacher JD, Myers RE (1986) Brain metabolic correlates of hypoxic ischemic cerebral necrosis in mid gestational sheep fetuses: significance of hypotension. J Cereb Blood Flow Metab 6:425–434
35. Vannucci RC, Lyons DT, Vasta F (1998) Regional cerebral blood flow during hypoxia-ischemia in immature rats. Stroke 19:245–250
36. Bejor R, Wozniak P, Allard M (1988) Antenatal origin of neurologic damage in newborn infants. Am J Obstet Gynecol 159:357–363
37. Scher MS, Belfar H, Martin J, Painter MJ (1991) Destructive brain lesion of presumed fetal onset: antepartum courses of cerebral palsy. Pediatrics 88:898–906