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# Prevention of mortality induced by perinatal asphyxia: Hypothermia or glutamate antagonism?

*Short Communication*

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**Summary.** Perinatal asphyxia was induced by keeping pups-containing uterus horns, removed by hysterectomy, in a 37°C or a 30°C water bath. Asphyxia for a period of 21–22 min at 37°C led to a 97% mortality within the first 20 min period following delivery. When the asphyctic period was extended to more than 22 min all the pups died following delivery. When the asphyxia was induced at 30°C, 100% of the delivered pups survived and were accepted by surrogate mothers. The protective effect of hypothermia could be observed even when the pups-containing uterus horns were exposed to a 45–46 min asphyctic period. Pretreatment with dizocilpine (0.2 mg/kg s.c.), or 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) (3–30 mg/kg s.c.), administered to the mothers one hour before hysterectomy, reduced slightly the mortality induced by a 21–22 min asphyctic period at 37°C. An increase in survival following a 22–23 min asphyctic period could only be observed after the highest dose of NBQX.

**Keywords:** Amino acids – Asphyxia – Hypothermia – Glutamate receptors – Rat

## Introduction

Acute perinatal asphyxia is a major cause of death and neurological injury in newborn babies. The incidence of asphyxia has been estimated as 2–4 per 1000 live term births, and has not decreased despite advances in perinatal and obstetric care (Hill, 1991; Younkin, 1992). Many babies die during the newborn period, and 20–30% of the survivors present long-term neurological deficits (Younkin, 1992). There is evidence that over-activation of excitatory amino acid receptors plays a role in the pathogenesis of perinatal hypoxic-ischemic brain injury, and therefore, it has been suggested that competitive and non-competitive

N-Methyl-D-Aspartate (NMDA), as well as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonists are useful as neuroprotective agents (Sheardown et al., 1990; Scatton et al., 1991; Barks and Silverstein, 1992).

We have developed a non-invasive animal model for studying the short- and long-term consequences of perinatal asphyctic lesions in rats, in conditions similar to those produced under labor in clinical situations (Bjelke et al., 1991). In this study we have analysed the short-term effects produced by asphyxia at 37°C, and the survival rate following hypothermia or glutamate antagonism.

## Material and methods

Asphyxia was induced in pups-containing uterus horns obtained by cesarean section on pregnant Sprague-Dawley rats. Female rats at the final day of gestation (b.w. 400–500g) were anaesthetised with halothane and hysterectomised. The entire uterus was taken out, the uterus horns containing the foetuses were detached and placed in a 37°C or a 30°C water bath for various periods of time (0–51 min) (control and asphyctic pups could be obtained from the same mother, since each mother produced approximately 15 pups). Following hysterectomy alone or asphyxia, the uterus horns were rapidly opened, the pups were removed and stimulated to breathe on a heating pad by tactile stimulation of the oral region with pieces of medical wipes. The umbilical cord was ligated and after a 60 min period the pups were given to the surrogate mothers. The time of asphyxia was measured from the time when the blood circulation to the uterus was cut off until the pups started to breathe.

In a series of experiments the non-competitive NMDA antagonist, dizocilpine ((+)-MK-801 hydrogen maleate; RBI, Natick, MA, USA) (dissolved in saline) (0.2 mg/kg s.c.) or the AMPA antagonist NBQX (Novo Nordisk A/S, Målov, Denmark, generously suplied by Dr L. Nordholm) (dissolved with a drop of NaOH in 5% glucose) (3–30 mg/kg s.c.) was administered to the mothers, in a single dose, 1 h before delivery.

Several parameters were acutely recorded by direct observation at a 40–60 min period following delivery: (1) survival, (2) gasping, and (3) colour of the skin. The reception by surrogate mothers and the survival for at least one month period were also recorded.

Comparisons on the rate of survival was tested by the non-parametric Cochran's test (Q). A level of $P < 0.05$ was considered critical for statistical significance.

## Results

Pups delivered from uterus horns removed by hysterectomy from rats at the final day of gestation, started regular breathing (respiratory frequency ≈60 per min) almost immediately after delivery was completed. These control pups showed a pink coloured skin and intensive vocalization and motility. They were accepted by surrogate mothers after a 60 min observation period. When the pups were accepted, they grew up in a manner similar to that shown by normally delivered rats (all the surviving rats reported in this studied were followed up for at least one month period).

Following a 15–16 min asphyctic period, induced in a water bath at 37°C (Table 1A) or 30°C (Table 1B), 100% of the pups started breathing short after delivery, they survived and were accepted by the surrogate mothers. Following a 19–20 min asphyctic period at 37°C (Table 1A), the pups had to be intensively stimulated to start to breathe. The surviving pups remained akinetic for a long period after delivery, showed a significant decrease in respiratory frequency (≈20 per min), which was accompanied of gasping, and showed a pink/pale skin. Approximately 30% of the pups died shortly after delivery. In contrast all the

**Table 1A,B.** Short-term effects of neonatal asphyxia performed under 37°C (**A**) and under 30°C (**B**)

| Treatment Time of asphyxia | Parameters S | | G | CS |
|---|---|---|---|---|
| Controls (M = 30; N = 30) | 100% | | No | Pink |
| A. Asphyxia at 37°C | | | | |
| 15–16 min (M = 10; N = 96) | 100% | | No | Pink |
| 19–20 min (M = 10; N = 100) | 78 ± 9% | * | 83% | Pink/Pale |
| 20–21 min (M = 10; N = 21) | 40 ± 16% | * | 88% | Pale |
| 21–22 min (M = 12; N = 100) | 3 ± 2% | * | 100% | Pale |
| 22–23 min (M = 10; N = 89) | 0% | * | 0 | 0 |
| 30–31 min (M = 6; N = 15) | 0% | * | 0 | 0 |
| 40–41 min (M = 4; N = 4) | 0% | * | 0 | 0 |
| 45–46 min (M = 5; N = 8) | 0% | * | 0 | 0 |
| 50–51 min (M = 4; N = 4) | 0% | * | 0 | 0 |
| B. Asphyxia at 30°C | | | | |
| 15–16 min (M = 3; N = 6) | 100% | | No | Pink |
| 19–20 min (M = 10; N = 50) | 100% | | 10% | Pink |
| 20–21 min (M = 10; N = 40) | 100% | | 10% | Pink/Pale |
| 21–22 min (M = 10; N = 40) | 100% | | 14% | Pink/Pale |
| 22–23 min (M = 5; N = 25) | 100% | | 20% | Pale |
| 30–31 min (M = 6; N = 15) | 100% | | 27% | Pale |
| 40–41 min (M = 6; N = 15) | 83 ± 12% | | 44% | Pale |
| 45–46 min (M = 5; N = 15) | 46 ± 5% | * | 100% | Pale |
| 50–51 min (M = 4; N = 15) | 0% | * | 0 | 0 |

*S* Survival (Means ± S.E.M.); *G* gasping, and *CS* colour of the skin. *M* number of mothers; *N* number of pups.
* $P < 0.05$, compared with controls

pups survived following a 19–20 min asphyctic period at 30°C (Table 1B). The pups started rapidly to breathe in a regular manner, although some initial gasping could be observed. The colour of the skin was similar to that in control pups.

The rate of survival rapidly decreased following prolonged asphyctic periods at 37°C (Table 1A), and as a whole, their physiological condition deteriorated. No pup survived following asphyctic periods longer than 22 min. In contrast, at 30°C (Table 1B), all the pups survived up to a 30–31 min asphyctic period, although some signs of physiological impairment (presence of gasping and pale skin) could be observed. Survival could still be observed following a 45–46 min asphyctic period. No survival was observed following asphyctic periods longer than 46 min.

**Table 2.** Effect of dizocilpine (0.2 mg/kg s.c.) and NBQX (10–30 mg/kg s.c.) (administered to the mother one hour before hysterectomy) on the short-term effects of neonatal asphyxia at 37°C. Controls are taken from the respective mother, but without undergoing asphyxia (see legend in Table 1).
* $P < 0.05$, compared with the saline group

| Treatment<br>Time of asphyxia | Parameters<br>S | | G | CS |
|---|---|---|---|---|
| SALINE | | | | |
| Control<br>(M = 5; N = 5) | 100% | | No | Pink |
| 21–22 min<br>(M = 12; N = 78) | 3 ± 2% | | 100% | Pale |
| 22–23 min<br>(M = 5; N = 10) | 0 | | 0 | 0 |
| DIZOCILPINE | | | | |
| Control<br>(M = 9; N = 9) | 100% | | No | Pink |
| 21–22 min<br>(M = 9; N = 42) | 19 ± 8% | | 86% | Pale |
| 22–23 min<br>(M = 9; N = 30) | 0% | | 0 | 0 |
| NBQX 10 mg/kg | | | | |
| Control<br>(M = 5; N = 5) | 100% | | No | Pink |
| 21–22 min<br>(M = 5; N = 31) | 16 ± 12% | | 100% | Pale |
| 22–23 min<br>(M = 5; N = 28) | 0% | | 0 | 0 |
| NBQX 30 mg/kg | | | | |
| Control<br>(M = 6; N = 9) | 100% | | No | Pink |
| 21–22 min<br>(M = 6; N = 20) | 21 ± 4% | * | 50% | Pale |
| 22–23 min<br>(M = 6; N = 23) | 18 ± 6% | * | 25% | Pale |

Table 2 shows the effect of pretreatment with saline, dizocilpine (0.2 mg/kg s.c.) or NBQX (10–30 mg/kg s.c.) (no effects were observed after NBQX 3 mg/kg s.c.), administered to the mothers, one hour before hysterectomy alone (controls) or hysterectomy followed by 21–22 or 22–23 min asphyctic periods at 37°C. Survival after a 21–22 min asphyctic period at 37°C was slightly increased by pretreatment with dizocilpine or NBQX. An increase in survival following a 22–23 min asphyctic period, at 37°C, could only be observed after the highest dose of NBQX (30 mg/kg s.c.).

## Discussion

A novel animal model for studying the consequences of perinatal asphyxia in rats is presented. The model is largely non-invasive and it mimicks conditions similar to those produced under labor in clinical situations (Bjelke et al., 1991). In this report we show the short consequences of various asphyctic periods, focusing on survival, which remains the major goal in any medical intervention.

We found that perinatal asphyxia, induced by immersing pups-containing uterus horns into a water bath at 37°C for a period longer than 22 min, led to a 100% mortality within the first 20 min period following delivery. When the uterus horns were kept in a 30°C water bath, 100% of the delivered pups started respiratory functions following tactile stimulation and were accepted by the surrogate mothers, even when the pups were exposed to a 30–31 min asphyctic period. All pups, however, died when the asphyctic period was extended to 50–51 min. The pups surviving prolonged asphyctic periods showed several signs of physiological impairment (e.g. decrease in respiratory frequency, motility and vocalization, and changes in the colour of the skin), however, they were accepted by surrogate mothers and survived for at least one month.

The striking protective effect of hypothermia shown in the present study is in agreement with the report by Ginsberg et al. (1992) demonstrating that low intraischemic brain temperature can protect brain neurons in rats subjected to transient forebrain ischemia, an effect probably due to reduction in brain energy demands and to a consequent decrease in the rate of ATP depletion (Young et al., 1983). The protective effect of hypothermia has been utilized world wide among primitive peoples and its effect on survival following asphyxia was pionerly studied by J.A. Miller and collaborators in the fifthies (see Miller, 1971).

It has been suggested that competitive and non-competitive NMDA, as well as AMPA antagonists are useful as neuroprotective agents in focal and global ischemia (Sheardown et al., 1990; Scatton et al., 1991; Barks and Silverstein, 1992). Thus, it was interesting to compare the protective effect of hypothermia with that of glutamate antagonists. In a series of experiments (Loidl et al. in preparation) it was found that dizocilpine given in doses above 0.5 mg/kg s.c., was lethal to the pups delivered by hysterectomy. A 100% survival was observed after 0.3 mg/kg s.c. of dizocilpine, however, they were not accepted by the surrogate mothers. However, following dizocilpine 0.2 mg/kg s.c. the pups were also accepted by the surrogate mothers. In the present study it was found that dizocilpine (0.2 mg/kg s.c.) could slightly increase survival induced by a 21–22 min asphyctic period at 37°C (from 3% to 19%). However, no protective effect

could be observed against longer asphyctic periods. In view of a report by Judge et al. (1991), demonstrating a protective effect of the AMPA antagonist NBQX, we also tested this drug in order to increase survival following asphyxia. NBQX, at the dose of 30 mg/kg s.c., increased survival following 21–22 min (from 3% to 21%) or 22–23 (from 0 to 18%) asphyctic periods (Table 2). No signs of protection were observed on pups suffering from longer than 23 min asphyctic periods (data not shown).

Thus, the present results suggest a preventive effect by the glutamate antagonists. The effect is, however, minor as compared with the prominent effect produced by hypothermia. Higher doses of dizocilpine are clearly toxic to the pups, and therefore cannot be tested. Higher doses of NBQX, as well as more specific and selective glutamate antagonists should be studied. However, whatever the results might be, the effects of the drugs should be compared with those produced by hypothermia.

In conclusion, a new non-invasive model for studying perinatal asphyxia is presented, which allows to study the short- and long-term consequences of hypoxic-ischemic lesions in rats, induced under conditions similar to those found under labor in clinical situations. Hypothermia appears to be superior to glutamate antagonism as a therapeutical intervention for increasing survival following perinatal asphyxia. Survival remains the most important goal of medicine, and therefore it should be considered when different therapeutical approaches are studied and should be compared with the effects of hypothermia.

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