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Hydrogen is neuroprotective and preserves cerebrovascular reactivity in asphyxiated newborn pigs

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Title: Hydrogen is neuroprotective and preserves cerebrovascular reactivity in asphyxiated newborn pigs

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Abstract

Hydrogen (H₂) has been reported to neutralize toxic reactive oxygen species (ROS). Oxidative stress is an important mechanism of neuronal damage after perinatal asphyxia. We examined if 2.1% H₂-supplemented room air (H₂-RA) ventilation would preserve cerebrovascular reactivity (CR) and brain morphology after asphyxia/reventilation (A/R) in newborn pigs. Anesthetized, ventilated piglets were assigned to one of the following groups: A/R with RA or H₂-RA ventilation (A/R-RA and A/R-H₂-RA; n=8,7), and respective time control groups (n=9,7). Asphyxia was induced by suspending ventilation for 10 min, followed by reventilation with the respective gases for 4 hours. After euthanasia, the brains were processed for neuropathological examination. Pial arteriolar diameter changes to graded hypercapnia [5-10%CO₂ inhalation] and NMDA, [10⁻⁴M]) were determined using the closed cranial window/intravital microscopy before and 1h after asphyxia. Neuropathology revealed that H₂-RA ventilation significantly reduced neuronal injury induced by A/R in virtually all examined brain regions including the cerebral cortex, the hippocampus, basal ganglia, cerebellum, and the brainstem. Furthermore, H₂-RA ventilation significantly increased CR to hypercapnia after A/R (% vasodilation was 23±4% *versus* 41±9%*, *p<0.05). H₂-RA ventilation did not affect ROS-dependent CR to NMDA. In summary, H₂-RA could be a promising approach to reduce the neurological deficits after perinatal asphyxia.

List of abbreviations:

A/R – asphyxia/reventilation

aCSF – artificial cerebrospinal fluid

CoBF – cortical blood flow

CR – cerebrovascular reactivity

H₂-RA – hydrogen-supplemented room air

MABP – mean arterial blood pressure

PAD – pial arteriolar diameter

RA – room air

ROS – reactive oxygen species

Introduction

Perinatal asphyxia induces brain injury that can result in death of the affected infants or severe neurodevelopmental deficits of the survivors. According to WHO estimates, perinatal asphyxia is responsible for 8% of the yearly 10.6 million deaths in children under the age of five years world wide (1). Thus it is important to seek suitable therapies that can increase survival and attenuate the neurological deficits. Despite the large number of affected infants, the incentives and involvement of the pharmaceutical industry has been limited so far perhaps due to financial concerns and ethical issues of initiating clinical trials in neonates (2). In fact, there is currently no effective neuroprotective pharmacotherapy to treat perinatal asphyxial encephalopathy. However, recently the Total Body Hypothermia for Neonatal Encephalopathy Trial (TOBY) study group has shown that moderate whole-body hypothermia improves outcome in full-term infants by decreasing the incidence of severe neurodevelopmental deficits, and thus body cooling is expected to become standard care very soon in this group of patients (3, 4).

Therapeutic hypothermia can be initiated up to six hours after birth, by the time the patient reaches a neonatal intensive care unit equipped to provide this care. Thus, there would be a window of opportunity for the employment of an immediate neuroprotective therapy until the neuroprotective cooling starts. The management of asphyxiated infants usually starts with resuscitation and involves mechanical ventilation. Reoxygenation of the tissues is a primary goal of resuscitation efforts, however, reoxygenation and reperfusion provides oxygen for the synthesis of reactive oxygen species (ROS) that can result in oxidative stress and subsequent reoxygenation/reperfusion injury (5-9). Resuscitation/ventilation with pure oxygen has been shown to be detrimental in neonatal large animal models (10-12), and in clinical trials as well

(13, 14). Resuscitation is now recommended with air to be supplemented with oxygen in infants with persistent central cyanosis (15). However, the gas mixture used for resuscitation could perhaps be used to deliver neuroprotective agents to the brain. Molecular hydrogen (H_2) has recently been shown to exert antioxidant properties (16). H_2 was indeed neuroprotective against hypoxic/ischemic injury in adult and neonatal rats (16-18), and also in a rat model of Parkinson's disease (19). However, the possible protective effect of H_2 against perinatal asphyxia has not been tested in a large animal model yet.

Therefore, the purpose of the present study was to investigate whether ventilation with H_2 -supplemented room air (H_2 -RA) preserves hypoxia-sensitive cerebrovascular reactivity (CR) and attenuates neuronal injury in asphyxiated newborn piglets. We also studied whether H_2 -RA ventilation in the absence of asphyxia/reventilation (A/R) stress affects CR or brain morphology.

Materials and Methods

Animals

Newborn (less than 1-day old) piglets of either sex (n=34) were delivered from a local farm to the laboratory on the morning of the experiments. All procedures were approved by the Animal Care and Use Committee of the University of Szeged. The piglets were anaesthetized with sodium thiopental (45 mg/kg ip, (Biochemie, Vienna, Austria) followed by intravenous application of α -chloralose (30-40 mg/kg iv, Sigma, St. Louis, MO, USA). Supplemental doses of α -chloralose (3-7 mg/kg/h) were administered to maintain a stable level of anesthesia determined by continuous monitoring the blood pressure and responsiveness to tactile stimuli.

The femoral artery and vein were catheterized to monitor arterial pH, blood gases and blood pressure, and to inject drugs and fluids, respectively. The animals were intubated through tracheotomy and artificially ventilated with RA (21% O₂, 79% N₂). The ventilation rate (~28/min) and tidal volume (~20ml) were adjusted to maintain physiological blood gas values. Core body temperature was kept in the physiological range (~38-39 °C) with electrical heating pads.

To determine CR, the piglets were equipped with a closed cranial window filled with artificial cerebrospinal fluid (aCSF) to measure pial arteriolar diameter (PAD) via intravital microscopy as described previously (20). Cortical blood flow (CoBF) was monitored using a two-channel laser-Doppler flowmeter (LDF, Periflux 4001, Perimed, Sweden) as described previously (12). In each experiment, a pial arteriole with a baseline diameter of 80-120 µm was selected to study CR. Ten-minute periods of arterial blood pressure and CoBF LDF data were recorded before, during, and after asphyxia, and at each hour of the reventilation period. Data were stored on a personal computer and were evaluated off-line using the Perisoft 1.30 software (Perimed).

Experimental protocol

The instrumented piglets were divided into four experimental groups 1) A/R with RA (21% O₂, 79% N₂) ventilation (A/R-RA; n=8), 2) A/R with H₂-RA (2.1% H₂; 21% O₂; 76.9% N₂) ventilation (A/R-H₂-RA; n=7), 3) time control group ventilated with RA (SHAM-RA; n=9); and 4) time control group ventilated with hydrogen-supplemented room air (SHAM-H₂-RA; n=7).

First, CR was determined to graded hypercapnia and NMDA. Graded hypercapnia was elicited by ventilating the animals with a gas mixture containing 5% CO₂, (21% O₂, balance N₂)

for 5-8 minutes, then with 10% CO₂ for additional 5-8 minutes. Ventilation was then switched back to RA, and PAD was allowed to return to baseline diameters. During a ten minute stabilization period the cranial window was gently flushed with aCSF, then NMDA dissolved in aCSF (10⁻⁴M, 5ml) was applied onto the cerebral cortex for 5-6 min, then the cranial window was repeatedly washed with aCSF. PAD was measured each minute during hypercapnia and NMDA stimulation, and the maximal vasodilation maintained for at least 2 min was used to determine CR to hypercapnia or NMDA, respectively.

After obtaining baseline CR values, asphyxia was induced (groups A/R-RA, A/R-H₂-RA) by suspending the artificial ventilation and clamping the endotracheal tube for 10 minutes. Reventilation started with the gas mixtures according to the protocol of the respective experimental groups. There was no need to apply chest compressions or drugs for resuscitation. In the SHAM groups, ventilation was switched to the respective gas mixtures after a RA ventilation time control period (10 min). After 1h ventilation, CR to hypercapnia and NMDA were again determined in all experimental groups, then ventilation was continued for an additional three hours. At the end of the 4h survival period, the anaesthetized piglets were euthanized with KCl (2 M, 10ml iv).

Neuropathology

An additional group of piglets (NAÏVE, n=3) were euthanized with sodium thiopental (150 mg/kg, ip) to provide an absolute control for the neuropathology studies. After euthanasia in all experimental groups, the brains were rapidly and carefully removed and immersion-fixed in 4% paraformaldehyde. Tissue blocks from the frontal, parietal, temporal and occipital cortices, the hippocampus, the cerebellum, the basal ganglia, the pons, and the medulla oblongata were embedded in paraffin, and sections were double stained with haematoxylin and eosin. Two

independent observers (O. Oláh and I. Németh), who were blinded to the treatments, quantitatively determined, then averaged the percentage of injured neurons in each region based on the presence of shrunken hyperchromatic (red) neurons with pyknotic nuclei.

Statistical analysis

Blood chemistry, mean arterial blood pressure (MABP), CoBF, and PAD data are expressed as mean \pm SEM, and have been analyzed using 2-way repeated measures ANOVA followed by the Student-Newman-Keuls *post hoc* test (SigmaStat, SPSS, Chicago, IL). Histopathology data were not normally distributed and were analyzed with Kruskal-Wallis ANOVA on Ranks followed by the Dunn's *post hoc* test for multiple comparisons. *p* values of <0.05 were considered statistically significant.

Results

Hemodynamics and blood chemistry

The MABP, pH and blood gas values were in the physiological range and did not differ significantly between the experimental groups before asphyxia (Table 1-2). Ventilation with gas mixtures containing 5% or 10% CO₂ to determine CR to hypercapnia resulted in graded increase in arterial pCO₂ with simultaneous drops in pH that were similar between all experimental groups before and after A/R injury (Table 1.). Asphyxia caused significant hypoxia, hypercapnia, acidosis, hypotension, and decrease in CoBF that were similar in both groups subjected to A/R (Table 1-2.). At the onset of reventilation, MABP rapidly increased then stabilized at near baseline values by the end of first hour (Table 2). MABP values were thus similar when determining CR to hypercapnia and NMDA before and after A/R. However, at 30

min and 1h after asphyxia, $p\text{CO}_2$ was significantly higher in the A/R-RA group compared to the A/R-H₂-RA group, albeit pH and $p\text{O}_2$ values were not significantly different (Table 1). In contrast, CoBF was ~20-25% lower in the A/R-H₂-RA group compared to the A/R-RA (Table 2). In the SHAM groups, the MABP remained in the normal ranges during the whole course of the experiment (Table 2). CoBF also appeared to be decreased by 15-20% in the SHAM-H₂-RA group compared to the SHAM-RA group, however, this difference did not reach statistical significance (Table 2).

H₂-RA preserves CR to hypercapnia after A/R injury

The first stimulation with graded hypercapnia elicited significant, dose-dependent, pial arteriolar vasodilation in all experimental groups (Figure 1A-B). Baseline PAD-s were not significantly different between repeated hypercapnia stimulations, their values were (first *versus* second stimulation): $88 \pm 4 \mu\text{m}$ *versus* $94 \pm 8 \mu\text{m}$ for group A/R-RA, $87 \pm 4 \mu\text{m}$ *versus* $92 \pm 7 \mu\text{m}$ for group A/R-H₂-RA, $85 \pm 4 \mu\text{m}$ *versus* $91 \pm 3 \mu\text{m}$ for group SHAM-RA, and $86 \pm 6 \mu\text{m}$ *versus* $82 \pm 7 \mu\text{m}$ for group SHAM-H₂-RA. A/R significantly attenuated CR to hypercapnia in the A/R-RA (Figure 1A) but not in the other experimental groups (Figure 1A-B). Thus, ventilation with H₂-RA under normoxic conditions in the SHAM-H₂-RA group did not affect CR to hypercapnia. Furthermore, ventilation with H₂-RA after asphyxia prevented the attenuation of CR to hypercapnia in the A/R-H₂-RA group (Figure 1A).

H₂-RA ventilation does not affect CR to NMDA

NMDA induced significant, repeatable pial arteriolar vasodilation in all experimental groups (Figure 1A-B). Thus, ventilation with H₂-RA did not affect CR to NMDA. Baseline

PAD-s were not significantly different between repeated NMDA applications, their values were (first *versus* second application): $87 \pm 4 \mu\text{m}$ *versus* $85 \pm 9 \mu\text{m}$ for group A/R-RA, $85 \pm 4 \mu\text{m}$ *versus* $91 \pm 6 \mu\text{m}$ for group A/R-H₂-RA, $86 \pm 4 \mu\text{m}$ *versus* $82 \pm 4 \mu\text{m}$ for group SHAM-RA, and $84 \pm 5 \mu\text{m}$ *versus* $80 \pm 6 \mu\text{m}$ for group SHAM-H₂-RA.

H₂-RA protects neurons after A/R injury

The percentage of damaged, red neurons was negligible in any brain region of the untreated control animals (NAÏVE animals): the median value was 0% in 7 regions, 2% in 3 regions and 3% in 2 regions (data not shown). In the time control animals (SHAM-RA, SHAM-H₂-RA groups), there was also only a minimal (1-4%) neuronal damage depending on the brain region studied, indicating that H₂-RA ventilation alone did not affect neuronal viability (Figure 2A-C, Figure 3). A/R significantly increased neuronal injury in all brain regions studied in the A/R-RA group. However, the increase in injured, red neurons was smaller in the A/R-H₂-RA group, showing a clear neuroprotective effect in virtually all brain regions examined (Figure 2A-C, Figure 3).

Discussion

The major findings of the present study are the following: ventilation with H₂-RA after asphyxia preserves CR to hypercapnia, and protects the brain from A/R-induced neuronal injury in piglets. Furthermore, H₂-RA ventilation *per se* does not affect CR or neuronal viability.

Oxidative stress induced by ROS produced during/after a hypoxic/ischemic episode is a widely accepted pathomechanism of perinatal brain injury (2, 21). Thus, administration of antioxidant drugs either to decrease ROS production or to neutralize ROS appears to be a simple,

straightforward approach to alleviate brain damage. However, the antioxidant drug needs to be present at the ROS level surge in the early reventilation/reperfusion exert its effect. For example, the xanthine oxidase inhibitor allopurinol was found ineffective when administered to severely asphyxiated term infants 4 hours after birth (22). The authors speculated that drug administration was too late to successfully target the immediate ROS surge following resuscitation. In fact, in a more recent study, the same research group has found that maternal treatment of *in utero* asphyxiated infants with allopurinol reduced the concentration of the brain injury marker S-100B in infants with therapeutic allopurinol levels (23). The disadvantage of maternal treatment is that placental transport of the drugs can vary greatly and the drug may not reach therapeutic levels in a significant portion of patients (23). H₂-RA ventilation could be an appealing technique for antioxidant neuroprotective therapy since administration could start simultaneously with the resuscitation effort to provide reoxygenation.

ROS also play physiological roles that have been demonstrated in numerous processes including neuronal and vascular signaling (24). Potent antioxidants may interfere with these physiological ROS-mediated mechanisms that can attenuate or even nullify their beneficial effects. Hydrogen gas has been suggested to selectively reduce the most toxic ROS: hydroxyl radicals and peroxynitrate anions (16), thus leaving the less deleterious/physiological ROS intact. Our present study provides evidence that H₂-RA ventilation does not affect a ROS-dependent neuronal-vascular response: NMDA-induced vasodilation. NMDA-induced vasodilation has been first described in the piglet (25), and CR to NMDA can be used to assess the integrity of the neurovascular unit (26). The mechanism of dose-dependent NMDA-induced pial arteriolar dilation and subsequent cortical hyperemia involves the neuronal synthesis and vascular actions of NO (20, 27). In the present study, the intact CR to NMDA during H₂-RA ventilation indicates

that H₂ does not interfere with the increases in NO levels necessary for the induction of pial arteriolar vasodilation. NMDA-induced vasodilation has been repeatedly shown to be sensitive to global cerebral ischemia/reperfusion, CR to NMDA (10⁻⁴ M) is attenuated by ~50% 1h after 10 min ischemia (26, 28). Interestingly, we have not observed a decrease in CR to NMDA in the present study after 10 min of asphyxia. The only study in the literature investigating the effect of A/R on NMDA-induced vasodilation reported “altered” CR to NMDA based on progressively increasing CR to NMDA in the reventilation period (29). However, in that study CR to NMDA was not determined prior to asphyxia. Furthermore, the vasodilation to 10⁻⁵M NMDA used in that study corresponds with our later findings with this dose of NMDA under normoxic conditions (20). Thus it is conceivable that A/R represents a less severe stress than global ischemia/reperfusion, and CR to 10⁻⁴M NMDA does not change appreciably using this stress.

However, A/R significantly attenuated CR to hypercapnia. In the piglet, hypercapnia-induced pial arteriolar vasodilation is dependent on the function of microvascular endothelial cells (20, 30). In the present study, H₂-RA ventilation preserved CR to hypercapnia after A/R indicating intact endothelial function. The preservation of CR likely results in more adequate microvascular blood flow matching neuronal needs and thus likely contributes to the neuroprotective effect of H₂-RA ventilation.

Our study is the first demonstrating the early neuroprotective effect of H₂-RA ventilation in a large animal model of perinatal asphyxia. We have previously shown the neurotoxic effect of 100% oxygen ventilation compared to RA ventilation after the same A/R stress used in the present study (12). The degree of neuronal injury was similar in the RA ventilated control groups in both studies, albeit in the present study we used a quantitative cell counting approach in contrast to the semiquantitative scoring system utilized in the previous study. Thus, our

simple A/R model of perinatal asphyxia is capable to detect both neuroprotective and neurotoxic effects related to the changes in the composition of gas mixture used for ventilation. We would like to emphasize that we observed a beneficial effect of H₂-RA ventilation in virtually all brain regions studied. In the previous study, the detrimental effects of 100% oxygen were significant only in the hippocampus and the cerebellum where reactive hyperemia during reventilation was twice higher than in the parietal cortex suggesting that enhanced oxygen delivery may exacerbate oxidative stress (12). We observed a mild decrease in CoBF during H₂-RA ventilation that may also contribute to its neuroprotective effect after A/R via limiting luxury oxygen delivery to the metabolically compromised brain parenchyma. The observed neuroprotective effect of H₂-RA ventilation of the present study is in accordance with previous data reporting neuroprotection by 2% H₂ gas in a neonatal rat hypoxia/ischemia model (18), and also in an adult rat stroke model (16).

The selection of gas used for the resuscitation of infants affected by perinatal asphyxia may represent an ideal way to implement an instant neuroprotective therapy. The importance of careful selection of inhaled oxygen concentration level is already well-established, and the present study confirms that addition of 2% H₂ gas may likely confer neuroprotection by combating ROS-inflicted neuronal damage during the early reperfusion/reoxygenation period. This concentration of H₂ (2-2.1%) is safe since H₂ has no risk of flammability below 4.7% in RA, and this H₂-RA ventilation protocol yielded H₂ concentration of ~20ng/ml (~10⁻⁵ M) in the arterial blood of adult rats suggesting an effective antioxidant capacity at this dose (16). Furthermore, other gases may potentially be added to the neuroprotective gas mixture to enhance neuronal survival and function. The anesthetic gas, xenon (50% inhaled concentration) has been found to augment neuroprotection afforded by hypothermia in the neonatal rat

hypoxia/ischemia model (31, 32), and helium (70% He/ 30% O₂) provided excellent neuroprotection in adult rat stroke models (33, 34).

The current study has several important limitations. The neuroprotective effect of H₂-RA ventilation was demonstrated in α -chloralose anesthetized piglets using only one A/R insult severity and one H₂-RA ventilation protocol. The anesthesia might enhance the neuroprotective potential of H₂-RA, and the neuroprotection afforded by H₂ can be diminished by increasing insult severity. H₂-RA ventilation likely exerts its greatest beneficial effect when administered immediately after reoxygenation, but it is unknown whether delayed administration of H₂-RA ventilation would have any protective effect. These clinically relevant topics warrant further studies to elucidate the full neuroprotective potential of H₂ perhaps in concert with other inert gases like helium, and therapeutic hypothermia.

In conclusion, H₂-RA ventilation after A/R provides early neuroprotection after asphyxia in newborn piglets, at least in part via preservation of neurovascular unit function. Ironically, after fruitless research for a “magic bullet” neuroprotective drug, the optimal combination of the simplest elements: hydrogen, helium, oxygen with appropriate body temperature control emerges as the most promising neuroprotective treatment in the management of perinatal asphyxia.

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Figure legends

Figure 1. H₂-RA ventilation preserves cerebrovascular reactivity to hypercapnia one hour after A/R. Graded hypercapnia induced by ventilation with 5-10% CO₂, and topical application of 10⁻⁴ M NMDA elicited similar, significant increases in pial arteriolar diameters in all experimental groups (Panel A-B). In time controls (Panel B), pial arteriolar responses were repeatable, and similar between the SHAM-RA (n=9, white bars) and the SHAM-H₂-RA (n=7, light gray bars) groups. Importantly, H₂-RA ventilation did not affect NMDA-induced, NO-dependent vasodilation (Panel B). After A/R, however (Panel A), pial arteriolar dilation to 10% CO₂ was significantly reduced in the A/R-RA group (n=8, black bars). However, in the A/R-H₂-RA (n=7, dark gray bars) group, pial arteriolar dilation to 10% CO₂ was significantly higher than in group A/R-RA, and not significantly different from the respective baseline response before A/R. p<0.05, * *versus* corresponding response before A/R, † corresponding responses after A/R in the A/R-RA *versus* the A/R-H₂-RA groups.

Figure 2. H₂-RA ventilation attenuates A/R-induced early neuronal injury. The percentage of damaged neurons was quantitatively determined at four hours after ten minutes of asphyxia, and in corresponding time control animals. The examined brain regions were: Panel A: CA1, CA2, CA3 and stratum granulosum (Str. Gr.) of the hippocampus; Panel B: frontal (Fr. Cx.), parietal (Par Cx.), temporal (Temp Cx.) and occipital (Occ. Cx.) cortices, Panel C: basal ganglia (Basal ggl.), pons, medulla, and cerebellum. Summated data from the hippocampal (ΣHippoc.) and cortical subregions (ΣCortex) are also shown on Panels A and B, respectively. The box plots show the median (horizontal line), the 25th-75th percentile (box), the 10th-90th percentiles (error

bars) and any outlying data points (bullets). The experimental groups are represented by gray, gray-hatched, white, and white hatched boxes for A/R-RA, A/R-H₂-RA, SHAM-RA and SHAM-H₂-RA (n=8,7,9 and 7), respectively. A/R resulted in significant increases in neuronal injury in virtually all examined brain regions in the A/R-RA but not in the A/R-H₂-RA group compared to the respective time controls. Thus, H₂-RA ventilation attenuated the detrimental effect of A/R on neuronal viability. $p < 0.05$, * *versus* corresponding time control (A/R-RA *versus* SHAM-H₂-RA and A/R-H₂-RA *versus* SHAM-H₂-RA) A/R, † A/R-RA *versus* the A/R-H₂-RA.)

Figure 3

Representative photomicrographs from the parietal cortex, the stratum granulare of the hippocampus, and the cerebellar cortex showing neuroprotection by H₂-RA ventilation. Shrunken, hyperchromatic (red) neurons with pyknotic nuclei are more common in samples obtained from animals of the A/R-RA group as compared with A/R-H₂-RA group. Time control groups (SHAM-RA, SHAM-H₂-RA) show minimal neuronal damage, the samples are similar to those obtained from untreated (NAÏVE) animals. Scale bar: 100 μ M.

Figure 1

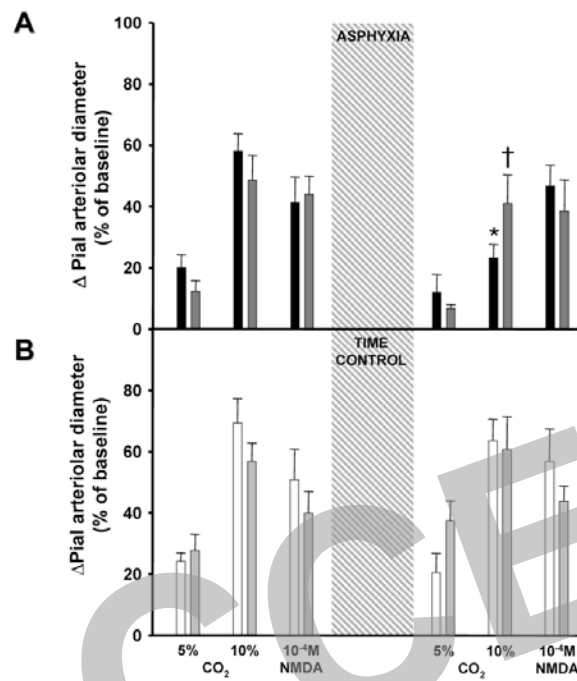


Figure 2

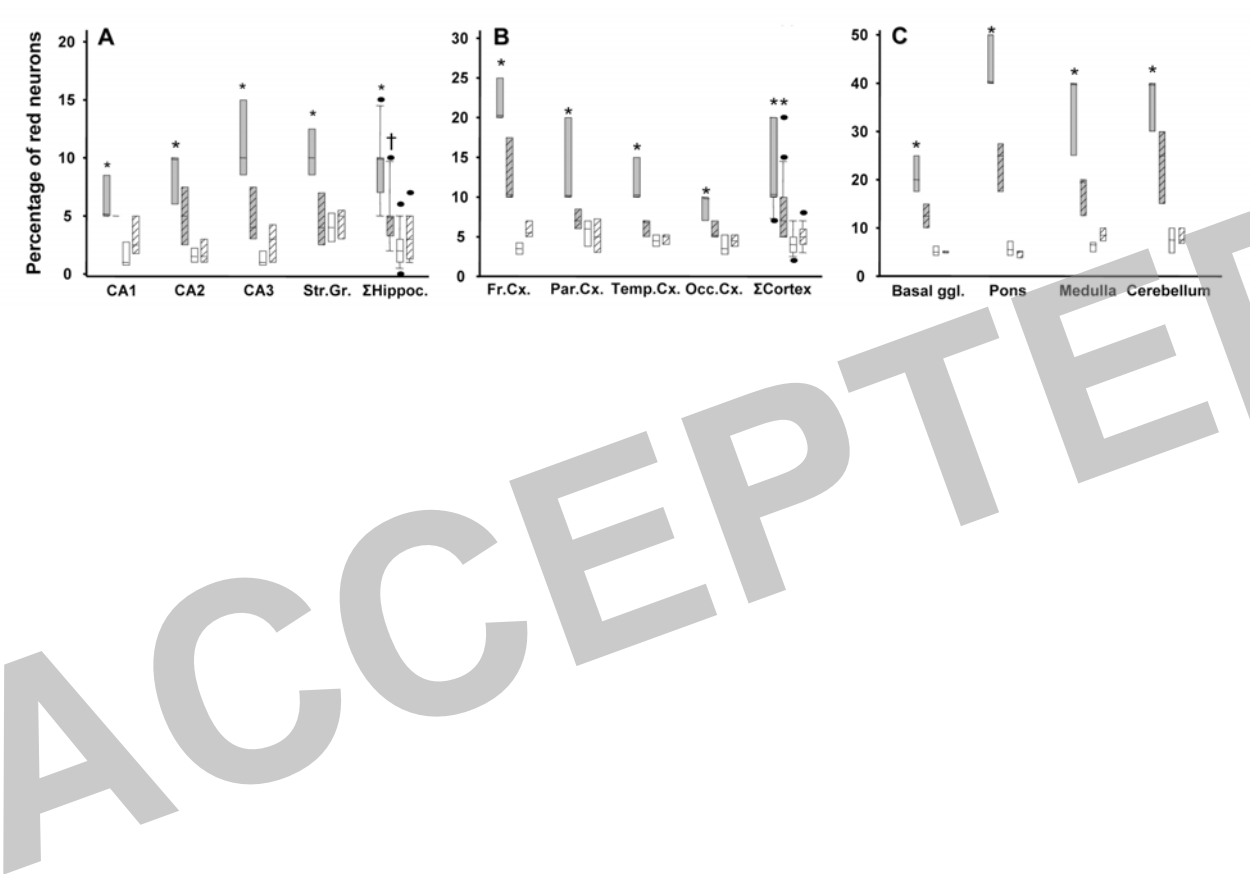
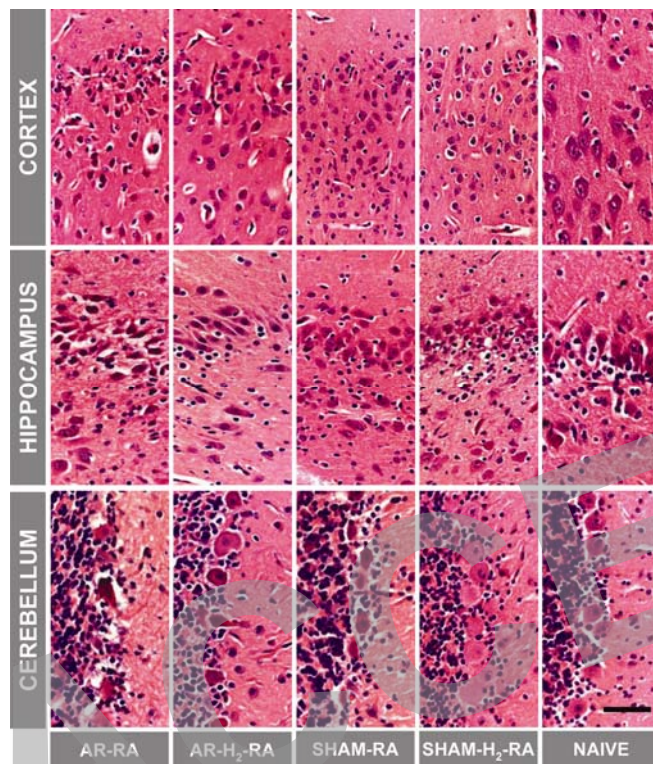


Figure 3



		Hypercapnia before asphyxia							Hypercapnia after asphyxia	
		Baseline	Asphyxia		Reventilation			Hypercapnia after asphyxia		
			5%CO ₂	10%CO ₂	10'	30'	1h	5%CO ₂	10%CO ₂	
A/R-RA (n=8)	pH	7,3±0,10	7,06±0,13	7,09±0,04	6,76±0,03	7,06±0,07	7,14±0,09	7,20±0,09	7,05±0,09	7,04±0,04
	pCO ₂ (mmHg)	29±1	51±6	80±5	133±10	59±8	43±5	56±9	71±12	90±11
	pO ₂ (mmHg)	82±4	86±4	85±5	11±1	66±8	61±5	60±8	70±9	70±13
A/R-H ₂ -RA (n=7)	pH	7,28±0,09	7,20±0,07	7,05±0,05	6,85±0,07	7,12±0,07	7,21±0,08	7,24±0,09	7,10±0,08	7,01±0,06
	pCO ₂ (mmHg)	31±4	48±5	77±5	123±12	44±5	32±4*	31±3*	54±5	82±3
	pO ₂ (mmHg)	71±4	81±4	86±3	5±0,4	69±6	66±4	68±4	78±5	77±3
SHAM-RA (n=9)	pH	7,53±0,02	7,32±0,03	7,15±0,02	N/A	7,45±0,06	7,46±0,05	7,44±0,06	7,30±0,03	7,19±0,04
	pCO ₂ (mmHg)	26±2	44±3	65±10	N/A	29±6	32±7	35±6	51±5	71±8
	pO ₂ (mmHg)	89±8	89±7	93±5	N/A	92±9	92±9	84±9	85±7	93±5
SHAM-H ₂ -RA (n=7)	pH	7,53±0,06	7,30±0,02	7,15±0,02	N/A	7,46±0,04	7,44±0,05	7,42±0,05	7,26±0,04	7,09±0,03
	pCO ₂ (mmHg)	33±2	51±5	85±6	N/A	34±3	35±4	37±6	57±3	85±5
	pO ₂ (mmHg)	78±3	75±4	66±7	N/A	74±7	75±9	70±7	77±6	70±12

Table 1.

*p<0.05 significantly smaller than corresponding values in group A/R-RA.

Table 2.

		Reventilation						
		Baseline	Asphyxia	10min	1h	2h	3h	4h
A/R-RA (n=7)	MABP (mmHg)	77±4	47±8	70±3	66±6	60±4	58±4	65±4
	CoBF (%)	100±0	31±7	83±4	107±15	101±9	103±5	105±7
A/R-H ₂ -RA (n=7)	MABP (mmHg)	74±5	41±12	70±3	51±3	63±5	55±4	57±4
	CoBF (%)	100±0	29±5	96±5	69±3*	81±6	76±8*	79±10*
SHAM-RA (n=9)	MABP (mmHg)	78±3	N/A	N/A	69±4	65±3	62±4	63±4
	CoBF (%)	100±0	N/A	N/A	119±19	121±22	108±15	117±21
SHAM-H ₂ -RA (n=7)	MABP (mmHg)	70±7	N/A	N/A	63±4	57±7	59±7	63±8
	CoBF (%)	100±0	N/A	N/A	93±5	80±8	80±8	85±11

*p<0.05 significantly smaller than corresponding values in group A/R-RA.