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Hypoxanthine, Xanthine, and Uric Acid Concentrations in Plasma, Cerebrospinal Fluid, Vitreous Humor, and Urine in Piglets Subjected to Intermittent *Versus* Continuous Hypoxemia

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ABSTRACT. Infants with sudden infant death syndrome have higher hypoxanthine (Hx) concentrations in their vitreous humor than infants with respiratory distress syndrome and other infant control populations. However, previous research on piglets and pigs applying continuous hypoxemia has not been able to reproduce the concentrations observed in infants with sudden infant death syndrome. To test whether intermittent hypoxemia could, in part, explain this observed difference, Hx, xanthine (X), and uric acid were measured in vitreous humor, urine, plasma, and cerebrospinal fluid in newborn piglets during intermittent hypoxemia (IH) or continuous hypoxemia (CH) of equal degree and duration. Urinary Hx excretion was significantly higher ($p < 0.04$) in the IH group after 60 min of hypoxemia. The vitreous humor Hx increase was significantly higher in the IH group (from 21.0 ± 7.8 to 44.1 ± 25.5 μmol/L, $p < 0.01$ *versus* baseline) than in the CH group (from 16.4 ± 4.2 to 23.2 ± 7.3 μmol/L, $p < 0.05$ *versus* baseline) ($p < 0.05$ IH *versus* CH). X increased significantly more ($p < 0.05$) in vitreous humor in the IH group than in the CH group. No differences between the two groups were found in plasma and cerebrospinal fluid for either Hx, X, or uric acid. We conclude that vitreous humor Hx and X increases more during IH than during CH. (*Pediatr Res* 34: 767–771, 1993)

Abbreviations

Hx, hypoxanthine
X, xanthine
Ua, uric acid
CSF, cerebrospinal fluid
SIDS, sudden infant death syndrome
IH, intermittent hypoxemia
CH, continuous hypoxemia

We have collected vitreous humor samples from different groups of pediatric patients for several years. These samples have been analyzed with regard to the purine metabolites Hx, X, and to some extent Ua. Hx, a well-established indicator of hypoxia (1–3), has been found to be elevated in 80% of SIDS victims compared with 20% in infants that die suddenly or unexpectedly from other causes (4). It was concluded in this study that SIDS, in most cases, is probably not a sudden event but may be preceded by a relatively long period of respiratory failure and hypoxia. However, previous experiments on piglets applying different degrees and durations of CH have not been able to reproduce equally high vitreous humor Hx values as observed in SIDS (5). Perhaps the higher Hx concentration formed in vitreous humor of SIDS victims is due to the fact that these infants suffer repeated episodes of hypoxemia before they die. In the present study, we wanted to determine whether IH or CH gives higher Hx concentrations. Hx has been shown to increase in hypoxemia in other body fluids like plasma, urine, and CSF (5–8). Concentrations of Hx, X, and Ua in plasma, CSF, urine, and vitreous humor were measured in two groups of pigs subjected to equal periods of hypoxemia either induced intermittently or continuously.

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MATERIALS AND METHODS

Approval. The experimental protocol was approved by the hospital's ethics committee for animal studies.

Animal preparations. Newborn piglets (2–5 d old, weight range 1530–2550 g, 18 male and eight female) were delivered by a local farmer on the day of the experiments. They are, from a CNS maturation point of view, close to human infants of 38 wk gestation based on histologic findings in the CNS (9). They were premedicated with azaperone (4 mg/kg intramuscularly), cannulated in a peripheral ear vein, and given atropine (0.025 mg/kg i.v.) and metomidate (4–10 mg/kg i.v.) until adequate anesthesia was achieved. Muscle relaxation was then induced with pancuronium bromide (0.2 mg/kg i.v.). Additional metomidate (4 mg/kg) was given when necessary. Pancuronium was not readministered. The pigs were intubated orally with a 3.0-mm cuffed Sheridan endotracheal tube (Sheridan Catheter Corp., Argyle, NY). The tube was connected via a small humidifier (Portex Termovent 600, Portex Limited, Hythe Kent, UK) to a Servo 900 B ventilator (Elema-Schønander, Stockholm, Sweden). The pigs were mechanically ventilated throughout the experiment at 40 breaths/min. Tidal volume was adjusted when necessary throughout the experiments to maintain arterial CO_2 between 4.6 and 5.9 kPa. Before hypoxemia, the pigs were ventilated with room air, and only pigs with arterial O_2 tension > 9.3 kPa were included in the experiment. The rectal temperature was monitored continuously and kept between 38.0 and 39.5°C with a heating blanket and when necessary a heating lamp. An arterial catheter was inserted proximally from a superficial artery on the inside of the hind leg so that ischemia to the leg was avoided. The catheter was connected to a strain-gauge transducer, and blood pressure was recorded continuously on a

Gould 2600S recorder (Gould Inc. Recording Systems, Cleveland, OH). The pulse was monitored continuously via skin electrodes. A midline suprapubic laparotomy was performed, a urinary catheter was placed in the bladder, and a lumbar puncture needle was placed in the cisterna magna. The pigs were placed on their side and allowed to stabilize for 30 min. During the experiments, they received via the ear vein an infusion of a solution containing 0.7% NaCl and 1.25% glucose at a rate of 10 mL/kg/h. Blood glucose was measured regularly with a reflectometer (Hypocount MX B, Boehringer, Mannheim, Germany), and the infusion altered as necessary to try to maintain glucose between 4 and 10 mmol/L.

Experimental protocol. The piglets were assigned to CH (n = 13) or IH (n = 13) groups. Animals in the CH group were ventilated with 8% O_2 in nitrogen, whereas animals in the IH group were given 8% O_2 in nitrogen for 10 min and 21% oxygen for 5 min repeatedly. The O_2 content of the inspired air was monitored with a Dameca OM 832 oxygen monitor (Dameca Inc., Copenhagen, Denmark).

Blood samples. Arterial blood samples were taken after surgery (−30 min), before hypoxemia, and then every 10 min in the CH group and every 10 and 5 min in the IH group. Temperature-corrected blood gases were measured with an AVL 945 Automatic Blood Gas System (AVL Biomedical Instruments, Schaffhausen, Switzerland). Blood for Hx analysis was collected into prechilled EDTA tubes and centrifuged rapidly for 10 min at 1800 × g, 4°C. Plasma was transferred to polypropylene tubes and frozen at −20°C until analysis. The withdrawn blood was replaced with an equal volume of sterile NaCl (0.9%) and the catheter flushed with heparinized saline (4 U/mL).

Sampling of CSF. Two hundred to 400 μL of CSF were drawn from the lumbar puncture needle before hypoxemia and then after 20, 40, and 60 min of hypoxemia. CSF was collected into polypropylene tubes as long as the needle was patent and then frozen at −20°C for later analysis.

Sampling of urine. The first urine sample was collected 30 min after surgery, and then samples were collected after every two periods of hypoxemia in the IH group and every 20 min in the CH group. Diuresis was measured and a 1-mL sample was frozen for later measurement of Hx and X. The concentration was related to diuresis, and the amount excreted per min per kg of body weight was calculated.

Sampling of vitreous humor. The conjunctiva was dissected free from the sclera. Vitreous humor was sampled with an empty Vacutainer (Vacutainer Systems Europe B.P., Maymeylan Cedex, France) through a puncture of the sclera about 6 mm posterior to the corneal limbus. Samples were taken only once from each eye before and after 60 min of hypoxemia. One hundred to 300 μL of fluid were taken at each sampling. The samples were immediately centrifuged for 10 min at 1800 × g, 4°C, to remove cells or other contaminating particles (pigmented pieces of retina). The clear vitreous humor was transferred into polypropylene tubes and frozen at −20°C until analysis.

Analysis of Hx, X, and Ua. The Hx and X levels in plasma, CSF, vitreous humor, and urine were analyzed using an HPLC method (10, 11) with modifications as previously described (5, 12). The coefficient of variation of the assay method was 0.75% based on seven separate analyses of the same sample throughout 1 d.

The Ua was analyzed using an MA 100/Uric Acid UV kit from Roche Diagnostic Systems in a COBAS BIO automated blood analyzer (Hoffmann-La Roche & Co., Ltd., Basel, Switzerland). Each sample was analyzed three times, and the mean values were used. The standard is Seronorm, batch 181 (Nycomed Pharma, Oslo, Norway).

Statistics. Values are given as mean ± SD. For vitreous humor and urine values, comparisons between groups were done with the Mann-Whitney U test and Wilcoxon matched pairs test as appropriate. For plasma and CSF values, statistical analyses were performed with two-way analysis of variance for repeated measures to determine the effects of time and group-time interaction. Paired or unpaired t tests with Bonferroni corrections were then performed. Analyses were performed with an SPCC/PC+ statistical package (SPSS Inc., Chicago, IL). A two-sided p value < 0.05 was considered significant.

RESULTS

Mean survival time of the IH group including reoxygenation time was 126 min (86 min of hypoxemia); the range was 89–168 min (59–118 min of hypoxemia). The mean survival time of the CH group was 82 min (range 57–109 min).

Blood gases and mean arterial blood pressure. Table 1 shows the arterial O_2 tension, base excess, and mean arterial blood pressure in the two groups of pigs before and after 20, 40, and 60 min of hypoxemia. No significant differences were observed between the two groups. However, each 5-min reoxygenation interval in the IH group produced a significant increase in mean arterial blood pressure ($p < 0.001$). The numbers are presented in Figure 1.

Hx, X, and Ua in vitreous humor. The concentrations of Hx in the vitreous humor of the two groups after 60 min of hypoxemia are given in Figure 2*A*. The increase was significantly higher in the IH group than in the CH group ($p < 0.05$). X also increased significantly more in the IH group (1.1 ± 0.2 to 3.1 ± 1.7 μmol/L) than in the CH group (1.0 ± 0.1 to 1.3 ± 0.4 μmol/L) ($p < 0.05$). Ua remained fairly stable. Mean values increased from 8.3 ± 2.9 to 8.7 ± 2.5 μmol/L in the IH group and from 8.4 ± 5.7 to 10.8 ± 5.5 μmol/L in the CH group.

Hx and X in urine. The output of Hx and X in the urine are given in Figure 2*B*. The Hx increase in the IH group was significantly higher ($p < 0.04$) than that in the CH group. No significant difference in X output was observed.

Table 1. *Blood gases, BE, and MABP (kPa) before and during IH (n = 13) and CH (n = 13)**

	Before hypoxemia	20 min hypoxemia	40 min hypoxemia	60 min hypoxemia
PaO_2				
IH	11.9 ± 1.3	3.7 ± 0.5	4.2 ± 0.6	4.6 ± 0.8
CH	11.0 ± 1.9	3.6 ± 0.6	3.9 ± 0.6	4.3 ± 1.3
BE				
IH	−1.1 ± 3.0	−9.5 ± 4.0	−16.0 ± 3.8	−21.0 ± 5.4
CH	−0.9 ± 3.9	−10.0 ± 4.3	−18.8 ± 5.1	−25.2 ± 5.4
MABP				
IH	9.8 ± 1.1	5.2 ± 1.3	3.6 ± 0.9	2.8 ± 0.9
CH	10.1 ± 1.2	4.8 ± 0.8	4.3 ± 0.8	2.8 ± 0.9

* Values are given as mean ± SD. PaO_2, arterial O_2 tension; BE, base excess; MABP, mean arterial blood pressure.

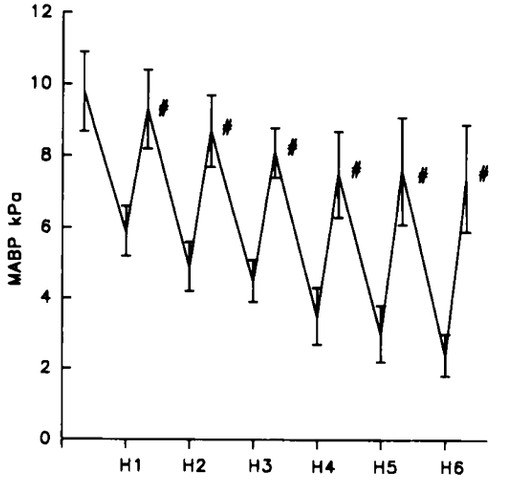

Fig. 1. Blood pressure (*MABP*) fluctuations in the IH group. End of each hypoxemia interval is indicated by *H1–H6* #, $p < 0.001$.

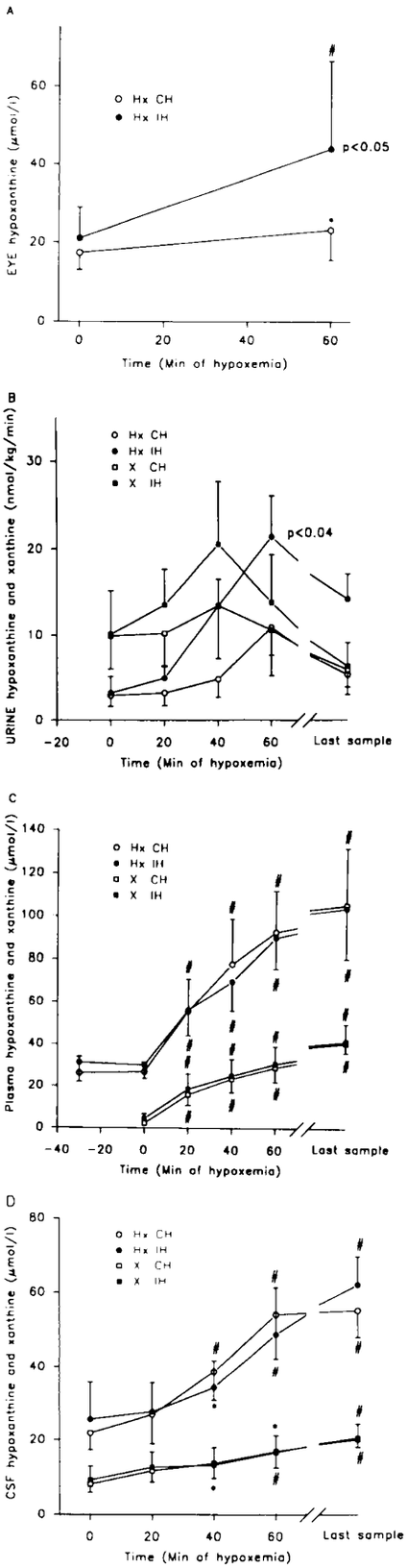

Fig. 2. *A*, Vitreous humor concentrations of Hx in the IH and CH groups. The Hx concentration increase in the IH group was significantly higher ($p < 0.05$) than in the CH group. #, $p < 0.01$ compared with values before hypoxemia. *, $p < 0.05$ compared with values before hypoxemia. *B*, The concentration is related to diuresis and is given as the amount excreted per min per kg body weight. No differences were observed with regard to the urinary output of X. Hx, however, was significantly higher ($p < 0.04$) in the IH group compared with the CH group. *C*, Concentrations of plasma Hx and X. #, $p < 0.01$ compared with values before hypoxemia. *, $p < 0.05$ compared with values before hypoxemia. *D*, Concentrations of CSF Hx and X. #, $p < 0.01$ compared with values before hypoxemia. *, $p < 0.05$ compared with values before hypoxemia.

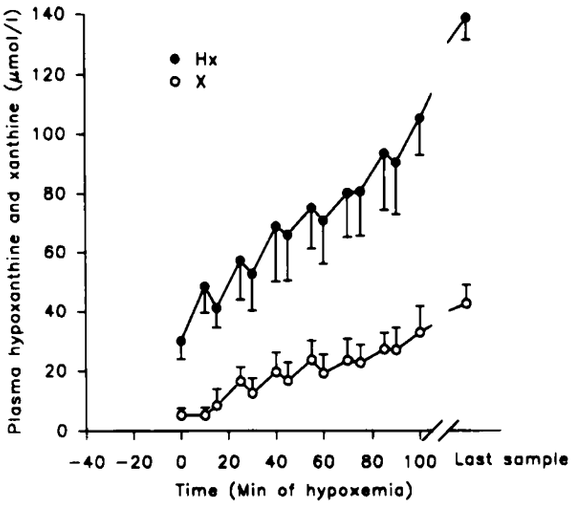

Fig. 3. The entire set of plasma samples in six piglets from the IH group. The fluctuations in the Hx and X increases can be observed with reference to the hypoxemic (10-min) and the reoxygenation (5-min) periods.

Hx, X, and Ua in plasma and CSF. The concentrations of Hx and X are given in Figure 2*D* and *C*, respectively. No significant differences could be demonstrated between the groups in either plasma or CSF. The entire set of plasma samples were analyzed from six piglets in the IH group and demonstrated the mode of Hx increase in plasma (Fig. 3).

Plasma Ua increased from 48.4 ± 11.2 to 74.5 ± 16.3 μmol/L in the IH group ($p < 0.01$ *versus* baseline) and from 44.2 ± 5.8 to 65.5 ± 12.9 μmol/L in the CH group ($p < 0.01$ *versus* baseline). In CSF, an increase from 3.0 ± 2.2 to 9.0 ± 5.9 μmol/L in the IH group and from 3.3 ± 1.1 to 5.9 ± 5.1 μmol/L in the CH group was observed. No significant differences could be demonstrated between the two groups for any metabolite in either plasma or CSF.

DISCUSSION

One rationale for using piglets in research involving hypoxemia and purine metabolism is that X oxidase is present in the lungs of rats, dogs, cats, and sheep but not of humans and pigs (13). When X oxidase is present in the lungs, Hx is cleared from the circulation even during severe hypoxemia. Furthermore, a good correlation between Hx, lactate, base deficit, and pH has been demonstrated in pigs (8, 14). On the other hand, in pigs as in most other mammals, Ua is not the end product of purine metabolism as it is in humans. For this reason, lower Hx, X, and Ua values compared with those in humans might be expected in body fluids of pigs.

Vitreous humor Hx increased 6.8 μmol/L in the CH group, and this was similar to the difference (4.6 μmol/L) demonstrated in a study in older pigs breathing 8% oxygen during a comparable time span (12). The significantly higher increase in the IH group indicates a more pronounced effect on the cellular energy charge of intermittent than chronic hypoxemia. No change in vitreous humor Ua was observed during hypoxemia, reflecting the lack

of X oxidase in vitreous humor and the fact that Ua penetrates poorly from plasma to vitreous humor.

Hx was probably not washed out from vitreous humor during the short intervals of reoxygenation in the IH group. Rather, the opposite was observed with a possible stepwise increase in vitreous humor Hx.

Plasma Hx more than tripled in both groups, whereas a doubling was observed in the CSF. The difference in X increase was even more pronounced with a close to 10-fold increase in plasma values compared with a doubling in CSF. Therefore, changes in CSF do not necessarily parallel those in plasma, as indicated by our results and supported by the findings of other researchers (15). One explanation for these differences observed in the two body fluids, may be the possibility of an active transport system in the choroid plexus (16). The direction of transport is not known. There are, however, speculations that this system acts to remove Hx from the CSF (17). Besides, only 40–70% of the CSF production is from the choroid plexus, whereas a substantial fraction (30–60%) is derived from the brain (18, 19). The brain contains higher levels of Hx guanine phosphoribosyltransferase than any other tissue (19). Therefore, in the brain, Hx is rapidly recycled into purine nucleotides instead of lost as unsalvageable purines.

We have to consider the renal handling of the purine metabolites in an attempt to explain the observed differences between Hx and X. The renal handling of X involves four steps: glomerular filtration, tubular reabsorption, active secretion, and postsecretory reabsorption, whereas Hx is eliminated mainly by filtration along with other purine bases, the first requiring a higher level of energy expenditure (20–22). There is a high renal clearance of Hx and it correlates with the creatinine clearance (22), whereas the renal clearance of X can exceed creatinine clearance, suggesting a tubular origin of at least some of the X (13). During hypoxemia, the most energy-dependent process will be the first to suffer, and at the end of hypoxemia the fall in blood pressure will result in reduced or even ceased renal perfusion.

Why would IH cause higher Hx in the vitreous humor and urine than CH? There seems to be a posthypoxic hypersensitivity to additional hypoxic stress as suggested by other researchers working with cats, fetal sheep, and gerbils (23–25). The piglets in the IH group had lived 40% longer at the time of the second vitreous sample due to a total of 25 min of reoxygenation. However, the increase observed in the IH group was more than 100% and cannot be explained by 25 min under normoxic conditions.

The systemic blood pressure during these 25 min of normoxia was considerably higher than during the time of hypoxemia. This probably explains the greater amount of Hx excreted in the kidneys and perhaps also a greater filtration of Hx from plasma to vitreous humor.

Whether Hx is produced locally in the eye or diffuses passively into the vitreous humor from plasma is not known. In one study (26), Hx in vitreous humor and plasma was significantly correlated ($r = 0.80$, $p < 0.001$). The last setting, however, was somewhat different from ours, with Hx infused i.v. under normoxic and normotensive conditions.

Vitreous humor contains mainly water (>99%) in which hyaluronic acid is dissolved. This solution is kept in a gel-like structure made up of collagen microfibrils, and the density is increasing from center to periphery. Hyalocytes are present in the periphery of the corpus vitreum and are responsible for both hyaluronic acid and collagen production. The actual vitreous humor is probably produced by diffusion and filtration from aqueous humor and the ciliary body and from the retinal capillary bed.

In our study, IH may have damaged the corpus ciliare and/or retinal capillaries causing leaky sites or at least increased the permeability to a greater extent than in the CH group. Oxygen radicals are produced during reoxygenation when Hx is metabolized to X and Ua by the enzyme X oxidase (8, 27), and they are potentially harmful molecules, having a direct damaging effect on cell membranes. In cerebral ischemia and reperfusion studies, increased transport of urea, sodium, and sucrose was demonstrated across the blood-brain barrier at reperfusion (28). With each reoxygenation (in the IH group), blood pressure increased significantly ($p < 0.001$) to more than the prehypoxemic level. This blood pressure increase lasted until the next hypoxemia was introduced. It is quite conceivable that this intermittent blood pressure increase will cause increased Hx filtration through capillary beds that may be increasingly damaged during the time span of the experiment. Furthermore, CH may not damage or change the permeability of the capillary beds to the same degree, inasmuch as the intermittent blood pressure changes were not experienced by the piglets in this group.

In conclusion, vitreous humor and urine Hx increases significantly more in IH than in CH.

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