Changes in Oxypurine Concentrations in Vitreous Humor of Pigs during Hypoxemia and *Post-Mortem*

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ABSTRACT. In the vitreous humor from three hypoxemic and one control group of pigs, hypoxanthine, xanthine, and uric acid concentrations were measured. The purine concentrations were measured before the hypoxemia, at the time of death, and 24 h *post-mortem*. During hypoxemia with arterial O_2 tension between 2.1 and 3.0 kPa [fractional inspired oxygen (FiO_2) = 0.08], hypoxanthine concentrations increased from a mean basal value of 11.7 ± 5.6 μmol/L to 16.3 ± 2.4 μmol/L at the time of death (NS). Xanthine concentrations changed from a basal value of 0.3 ± 0.1 μmol/L to 0.6 ± 0.2 μmol/L ($p < 0.02$), and uric acid changed from 3.4 ± 1.1 μmol/L to 5.0 ± 4.5 μmol/L (NS). During hypoxemia with arterial O_2 tension between 3.0 and 4.0 kPa (FiO_2 = 0.11), hypoxanthine increased in the vitreous humor from a mean basal value of 9.1 μmol/L to 20.3 μmol/L at the time of death ($p < 0.02$). Xanthine concentrations increased from 0.3 μmol/L to 1.3 μmol/L ($p < 0.05$), whereas there was no change in uric acid concentration (basal 5.0 ± 0.8 μmol/L and final 4.5 ± 1.0 μmol/L). During milder hypoxemia with arterial O_2 tension between 4.3 and 5.6 kPa (FiO_2 = 0.14), or in the control group (FiO_2 = 0.21), neither of the metabolites changed significantly. The vitreous humor was not stable *post-mortem*, inasmuch as the mean concentration of hypoxanthine increased from 18.2 ± 7.7 μmol/L to 121.6 ± 57.4 μmol/L 24 h *post-mortem* ($p < 0.01$). In the same period, xanthine increased from a mean 0.9 ± 0.6 μmol/L to a mean 4.9 ± 5.2 μmol/L ($p < 0.01$). (*Pediatr Res* 28: 482–484, 1990)

Abbreviations

SIDS, sudden infant death syndrome
CSF, cerebrospinal fluid
PaO_2, arterial O_2 tension
FiO_2, fractional inspired oxygen

The etiology and pathogenesis of SIDS is still unknown. Some authors claim that SIDS might be due to an acute process, such as cardiac arrhythmia (1), whereas others find evidence of a more prolonged disease process, which may include long-standing hypoxia (2, 3). During tissue hypoxia, elevated hypoxanthine levels have been found in plasma (4–6), CSF (7, 8), and urine (9). We have also measured the hypoxanthine levels in vitreous humor *post-mortem*, and documented high levels when death was preceded by respiratory failure and hypoxemia (10, 11). Recently, we reported high levels of hypoxanthine in the vitreous humor of victims of SIDS (12), and therefore concluded that hypoxemia precedes death in SIDS.

In our present study, we report how three degrees of hypoxemia in young pigs influence the level of hypoxanthine, xanthine, and uric acid in the vitreous humor. Furthermore, we investigated the time factor involved in hypoxanthine accumulation in the vitreous humor during hypoxemia. We also report how hypoxanthine concentrations in the vitreous humor change during the first 24 h *post-mortem*. We found this important, inasmuch as the time from death to sample collection could have influenced the concentrations of hypoxanthine in the vitreous humor that we have reported from our SIDS cases.

MATERIALS AND METHODS

Approval. The local hospital's ethical committee for animal studies approved these experiments.

Animal model. The animal model was similar to that previously described (13). Twenty-two young pigs of either sex (the males had been castrated shortly after birth) weighing 17–22 kg (mean 18.1 kg) were used. They were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg). An i.v. line was established through an ear vein, and a continuous infusion of Ringer acetate (10 mL/kg/h) was given during the experiment. A further 100 mg of sodium pentobarbital was given i.v. if necessary every 30 min. A cuffed tube was placed in the trachea via a tracheostomy. Artificial ventilation was given by a ventilator (Servo-ventilator 900B, Elema-Schønander, Stockholm, Sweden). Ventilation rate and tidal volume were adjusted until arterial CO_2 tension ranged between 4 and 5.3 kPa (30–40 mm Hg). No further adjustments of the ventilator were done during the hypoxemic period.

Polyethylene catheters were inserted into a femoral artery and a femoral vein. The arterial catheter was used for measuring blood gases. The venous catheter was used for giving Ringer acetate plus additional sodium pentobarbital.

After surgery, the pigs were left for a stabilizing period of at least 1 h before basal concentrations were measured, and then divided into three hypoxemic and one control group. Seven animals were made hypoxemic by artificial ventilation with 8% oxygen in nitrogen (FiO_2 = 0.08) (group 1). Seven animals were ventilated with 11% oxygen (FiO_2 = 0.11) (group 2). Five animals were ventilated with 14% oxygen (FiO_2 = 0.14) (group 3), and

three animals were ventilated with room air (FiO$_2$ = 0.21), serving as normoxemic controls (group 4). The oxygen concentrations were measured continuously in all pigs during the experiments with a Dameca OM 831 oxygen monitor (Dameca Inc., Copenhagen, Denmark).

During hypoxemia with FiO$_2$ = 0.08, the PaO$_2$ remained between 2.1 and 3.0 kPa (15.8–22.5 mm Hg). This degree of hypoxemia lasted until the animals died after a mean of 170 min (range 120–240 min). During hypoxemia with FiO$_2$ = 0.11, the PaO$_2$ remained between 3.0 and 4.0 kPa (22.5–30 mm Hg), and these pigs died after a mean of 283 min (range 165–445 min). During the mildest hypoxemia (FiO$_2$ = 0.14), the PaO$_2$ remained between 4.3 and 5.6 kPa (32.5–42 mm Hg). All animals tolerated this degree of hypoxemia for 480 min, after which they were killed with an i.v. overdose of sodium pentobarbital. The pigs in the control group were observed from 185 to 480 min and then killed. During artificial ventilation, 1 mL of pancuronium bromide (Pavulon 2 mg/mL, Organon Teknika, Boxtel, Netherlands) was given i.v. every 30 min to prevent muscular contractions, which may lead to artificially high hypoxanthine levels (14).

Blood samples. Arterial blood gases were measured with an AVL 945 Automatic Blood Gas System (AVL Biomedical Instruments, Schaffhausen, Switzerland) before hypoxemia, during hypoxemia at 5, 15, and 30 min, and then every 30 min as long as the experiment lasted. The withdrawn blood was immediately replaced with an equal volume of sterile NaCl (0.9%).

Sampling of vitreous humor. In the pig's right eye, the conjunctiva was dissected free from the sclera. Any bleeding was stopped by microcoagulation (Concept Cauterette I, Concept Inc., Clearwater, FL). The vitreous humor was sampled twice with a vacutainer through a puncture of the sclera about 6 mm posterior to the corneal limbus. Two hundred to 400 μL of fluid were taken at each sampling. The two sampling times were before start of the hypoxemia and just after death. Multiple sampling during the hypoxemia was tried, but this procedure led to artificially high values of hypoxanthine compared with control values from the left eye (see below) and the procedure had to be abandoned (results not shown).

Within 5 min of the animal's death, a similar procedure was done in the left eye to obtain a control sample of vitreous humor. In case of a difference in the hypoxanthine concentration in the two eyes at the time of death, the value from the unpunctured (left) eye was accepted. In some animals, the left eye was then enucleated, put in a glass jar and stored at 4°C. Twenty-four h *post-mortem*, a second sample from the vitreous humor of this eye was taken for analysis of hypoxanthine and xanthine.

The samples of vitreous humor 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 the time of analysis.

Analysis of hypoxanthine, xanthine, and uric acid concentrations. The hypoxanthine and xanthine levels in vitreous humor were analyzed using an HPLC method (15, 16).

The sample of vitreous humor had to be diluted before filtration at least three times with the mobile phase (0.005 M KH$_2$PO$_4$, pH 5.7). Using a syringe, the vitreous humor was passed through a 4-mm Millex-HV$_4$ unit with 0.45-μm pore size (Millipore Corp., Bedford, MA).

The chromatography, identification, and quantification of the peaks in the HPLC effluents were done as previously described (13). All pure standards used for the HPLC were obtained from Sigma Chemical Co., St. Louis, MO.

Uric acid was measured on an RA-1000 (Technicon Instruments, Tarrytown, NY) automatic blood analyzer.

Statistics. Mean values ± SD are given. Wilcoxon's two-tailed rank test was applied for testing the significance of differences between comparable groups. A p value < 0.05 was considered statistically significant.

RESULTS

Hypoxanthine, xanthine, and uric acid in the vitreous humor. The concentrations of hypoxanthine in the vitreous humor of the four groups are given in Figure 1. The mean concentration before the start of hypoxemia was 10.3 ± 4.0 μmol/L, with no statistical difference between the groups. In group 1, the concentrations increased only slightly up to the time of death [final level 16.3 ± 2.4 μmol/L (NS)], giving an average change per hour hypoxemia of 1.7 ± 2.9 μmol/L/h (NS). However, in group 2, the concentration of hypoxanthine in the vitreous humor increased up to the time of death in all the pigs, with a mean augmentation of 12.0 ± 7.9 μmol/L, ($p < 0.02$) and an average rate of change of 3.4 ± 3.3 μmol/L/h ($p < 0.02$).

In the mildest hypoxemic pigs, group 3, the increase in the concentration of hypoxanthine in the vitreous humor (4.6 ± 7.3 μmol/L) was not significant. The average rate of change per hour of hypoxemia (0.6 ± 0.9 μmol/L/h) was significantly lower than that in group 2 ($p < 0.02$). There were no changes in the concentration of hypoxanthine in the vitreous humor in the control group.

The mean xanthine concentration in the vitreous humor in the pigs was 0.3 ± 0.1 μmol/L before hypoxemia. In group 1, it increased to 0.6 ± 0.2 μmol/L ($p < 0.02$), and in group 2 it increased to 1.3 ± 0.7 μmol/L ($p < 0.05$), whereas in the two other groups there was no significant increase.

The mean uric acid concentration in the vitreous humor of pigs in groups 1 and 2 was 4.1 ± 1.3 μmol/L before hypoxemia. There was no change in uric acid after hypoxemia; in group 1, the final concentration was 5.0 ± 4.5 μmol/L (NS), and in group 2 the final concentration was 4.5 ± 1.0 μmol/L (NS). Uric acid was not measured in the two other groups.

Eleven randomly selected left eyes from all four groups of pigs were enucleated shortly after death and stored at 4°C, and a second sample of vitreous humor was collected from these eyes 24 h later. Figure 2 shows that the concentration of hypoxanthine in these eyes changed from a mean of 18.2 ± 7.7 μmol/L at the time of death to a mean of 121.6 ± 57.4 μmol/L at 24 h after death ($p < 0.01$). The average rate of change in hypoxanthine concentration in the vitreous humor of pigs *post-mortem* at 4°C was 4.3 ± 2.3 μmol/L/h. The postmortem change was independent on the degree of hypoxemia before death.

The concentration of xanthine in these eyes changed from a mean of 0.9 ± 0.6 μmol/L at the time of death to a mean of 4.9 ± 5.2 μmol/L at 24 h after death ($p < 0.01$).
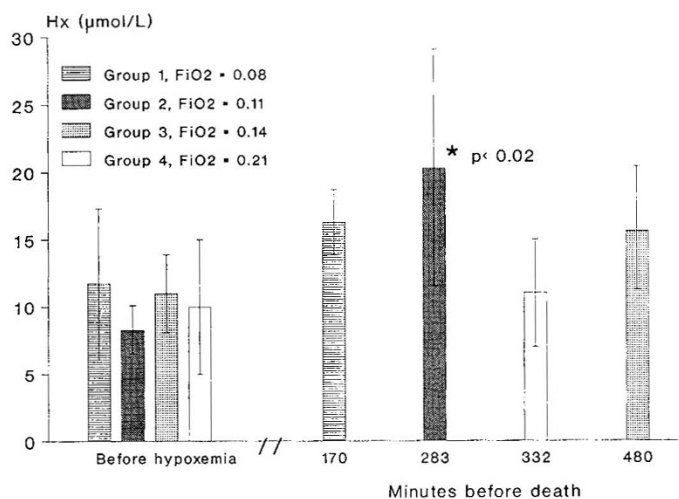
Uric acid was not measured at 24 h *post-mortem*.

Fig. 1. Hypoxanthine (*Hx*) concentrations (μmol/L) in the vitreous humor before the start of hypoxemia and at the time of death in four groups of pigs. Mean time until death varied in the four groups. Mean values ± SD are given. *$p < 0.02$ compared with values before hypoxemia.
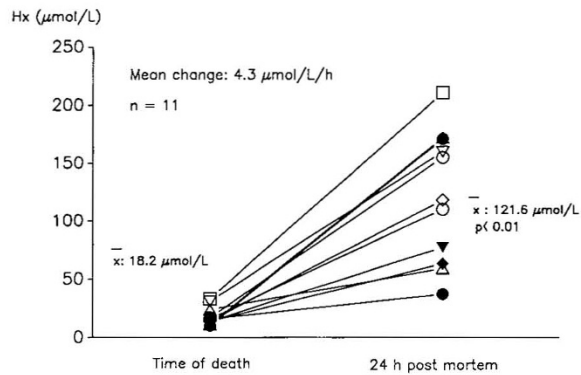
Hx (μmol/L)

Fig. 2. Hypoxanthine (*Hx*) concentrations (μmol/L) in the vitreous humor at the time of death and 24 h *post-mortem* in 11 eyes randomly selected from pigs from all four groups of hypoxemia. Storage temperature was 4°C.

DISCUSSION

Vitreous humor is easily sampled *post-mortem* and is considered to be relatively free from contamination by blood, bacteria, and products of postmortem autolysis. During life, however, vitreous humor is difficult to obtain, and to our knowledge, the biochemical changes occurring before and after death in the same individuals have not previously been studied.

In an earlier report on postmortem hypoxanthine levels in the vitreous humor (10), we assumed that values found in cases of sudden death reflected the levels *in vivo*, ranging between 0 and 540 μmol/L, with a mean 10 to 20 times higher than in plasma. Our present results show that this assumption was wrong, as the vitreous humor of pigs was found to have a basal concentration of hypoxanthine even below that found in plasma or CSF (13). Our present findings correspond well with the data recently published by Gardiner *et al.* (17).

Subjecting our pigs to severe hypoxemia (FiO$_2$ = 0.08) did not lead to any significant increase in vitreous humor hypoxanthine. However, subjecting the animals to a milder degree of hypoxemia (FiO$_2$ = 0.11) increased the mean vitreous humor hypoxanthine concentration about 2.5-fold. The duration of the hypoxemia in group 2 was, however, much longer (283 *versus* 170 min in group 1). These results suggest, therefore, that for reaching high concentrations of hypoxanthine in the vitreous humor, the length of the hypoxemic period may be as or perhaps more important than the degree of hypoxemia. Our present experiment supports our belief that longstanding hypoxemia is necessary to raise the hypoxanthine levels in the vitreous humor up to the values found in SIDS cases or in children dying of respiratory distress syndrome (11, 12).

Whether hypoxanthine and xanthine are formed locally in the tissues of the eye during hypoxemia or they diffuse passively into the vitreous humor from the CSF or plasma, we do not know. In these pigs, no relationship was found between the concentrations in the vitreous humor and concentrations measured in CSF or plasma (13), except that a lower concentration was always found in the vitreous humor than in the other two extracellular body fluids.

However, previous studies on uric acid concentrations in monkeys and rats (18–20) indicate that the concentration of uric acid in the aqueous humor and in the retina depends on local synthesis, rather than on a systemic increase in the concentration. Our findings of no change in uric acid concentrations in the vitreous humor after hypoxemia indicate that under these conditions no uric acid is formed. This could only mean that the enzyme xanthine oxidase (EC 1.1.3.22) is not present in the eye or vitreous humor in any significant concentration.

The vitreous humor hypoxanthine concentrations are not stable *post-mortem*. We have recently measured human vitreous humor hypoxanthine serially *post-mortem* (Rognum TO, Poulsen JP, Hauge S, Øyasæter S, Saugstad OD, unpublished data). When the bodies were stored at 6°C, there was an average increase of 3.5–4.0 μmol/L/h. This change corresponds well with the increase of 4.3 μmol/L/h *post-mortem* in pigs reported in this paper, but is considerably lower than the findings recently reported by Gardiner *et al.* (17). He found an hourly postmortem increase in pigs of about 13 μmol/L, but his storage temperature was 20°C. Gardiner's data thus indicate that hypoxanthine concentration increases faster in the vitreous humor with higher surrounding temperature.

Whether the enucleation of the eyes, or the fact that these eyes had been punctured once at the time of death, influences the concentration measured 24 h *post-mortem* is not known.

In summary, postmortem vitreous humor hypoxanthine concentrations may reflect the duration of hypoxemia before death better than the degree of hypoxemia. Further, this may also be true in human newborns where no significant increase in vitreous humor hypoxanthine was found unless the baby had suffered from at least 4 to 5 h hypoxemia before death (11).

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