J exp. Biol. 159, 501–505 (1991)
Printed in Great Britain

SHORT COMMUNICATION

INFLUENCE OF PREGNANCY ON THE OXYGEN AFFINITY OF RED CELLS FROM THE NORTHERN PACIFIC RATTLESNAKE *CROTALUS VIRIDIS OREGANUS*

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Accepted 30 April 1991

Fetal blood has a higher affinity for oxygen than maternal blood in almost all vertebrates examined. This difference appears to be due to the characteristics of fetal blood rather than to changes in the blood of the adult. Recently we reported that pregnancy in the garter snake *Thamnophis elegans* is associated with a significantly lower oxygen affinity of red cells in the adult (Ingermann *et al.* 1991). The effect of pregnancy appears to be due to an increase in the concentration of nucleoside triphosphate (NTP), primarily adenosine triphosphate (ATP), in the maternal red cells. ATP lowers the oxygen affinity of *T. elegans* hemoglobin (Berner and Ingermann, 1988). This was the first time pregnancy had been reported to induce an appreciable change in the oxygen affinity of the red cells of the mother.

It is not clear whether this effect of pregnancy is restricted to a single snake species (in the family Colubridae), or is a more common mechanism among viviparous reptiles to facilitate oxygen delivery to the fetus. Consequently, we measured the NTP concentration and oxygen affinity of red cells from pregnant and nonpregnant female, male and fetal northern Pacific rattlesnakes *Crotalus viridis oreganus* (Holbrook) (Viperidae).

Consistent with the effect of temperature on oxygen affinity of reptilian blood (Pough, 1969; Greenwald, 1971; MacMahon and Hamer, 1975), the red cell oxygen-affinity of the garter snake was significantly lower at 34°C, a typical day temperature, than at 20°C, a typical night temperature (Ingermann *et al.* 1991). In this snake the effect of pregnancy on the oxygen affinity of the adult red cell was apparent at both 20 and 34°C. To establish the effect of pregnancy on the oxygen affinity of adult red cells from the rattlesnake at approximate day and night temperatures, we analyzed oxygen affinities at 20 and 34°C.

Rattlesnakes were collected from hibernacula during the early summer of 1989 and 1990, in Latah and Nez Perce Counties, Idaho. Snakes were identified as *Crotalus viridis oreganus* according to Nussbaum *et al.* (1983). The sex of the snakes was determined by probing for the hemipenes and pregnancy was

Key words: oxygen affinity, pregnancy, reptile, ATP, snake, *Crotalus viridis oreganus*.

determined by abdominal palpation. The developmental stage of the fetuses was determined according to morphological characteristics described by Zehr (1962). Fetuses of developmental stages 30–37 were examined in this study. (Stage 37 is the last prenatal stage.)

Adult snakes were bled by puncturing the caudal vein posterior to the vent with a heparinized 23 gauge needle, and the blood was immediately transferred into ice-cold heparinized buffer at pH 7.4 (143 mmol l^{-1} NaCl, 3 mmol l^{-1} KCl, 1.5 mmol l^{-1} $CaCl_2$, 20 mmol l^{-1} Tris, pH adjusted with HCl). To obtain fetal snakes, the pregnant female was cooled in ice for 15 min, decapitated and dissected. Fetal red cells were collected by sectioning the fetuses in ice-cold heparinized buffer at pH 7.4 and then filtering the suspension through glass wool to minimize debris. The cells were washed three times in this saline by centrifugation at 1000 *g* for 5 min at 4°C.

To determine red cell oxygen-affinity, red cells were suspended in the buffer at pH 7.4 and a hematocrit of 10–18 %. Oxygen-affinity measurements were conducted by the method of Tucker (1967) with a TC500 Tucker cell and model 781 oxygen meter from Strathkelvin Instruments (Glasgow, Scotland) as previously described (Ingermann *et al.* 1991). Cells were incubated at 20 or 34°C with a mixture of nitrogen and compressed air. The gas flows were manually adjusted and kept constant with Cole-Parmer (Chicago, IL) 150 mm variable-area flowmeters. The oxygen content of the gas mixture was continuously monitored with a model 57 oxygen meter and a 5739 oxygen probe from YSI Inc. (Yellow Springs, Ohio). P_{50} values were determined from Hill plots, each using 8–10 data points corresponding to a red cell suspension that was 20–80 % saturated with oxygen.

To determine red cell NTP concentration, the red cell suspension was extracted with an equal volume of ice-cold 12 % trichloroacetic acid and centrifuged for 2 min in a Fisher microcentrifuge (model 235C). Acid extraction of the cells was completed within 30 min of blood collection. The NTP concentration of the supernatant fraction was determined using an enzymatic assay kit for ATP (no. 366-UV) from Sigma Chemical Co. (St Louis, MO). Red cell NTP concentration, in mmol l^{-1}, was calculated from the NTP concentration in the supernatant divided by the hematocrit of the suspension. The hemoglobin concentration of the red cell suspension was determined spectrophotometrically as the cyanmethemoglobin derivative using the millimolar heme extinction coefficient of 11.0 at 540 nm. NTP concentrations are also expressed as mole NTP per mole hemoglobin tetramer.

Each *N* value for fetal red cell oxygen-affinity and NTP concentration represents blood collected from fetuses from a single litter. All data are presented as the mean±S.D. Statistical analyses were based on a single-factor analysis of variance (ANOVA) followed by the Tukey test (alpha=0.05) for equal or unequal sample sizes, as appropriate (Zar, 1984). The variances within treatment groups were not significantly different.

Fetal rattlesnake red cells had significantly higher affinities for oxygen (i.e. lower P_{50} values) compared with maternal red cells at 20 and 34°C (Table 1).

Table 1. *P_{50} (kPa) at 20 and 34°C and nucleoside triphosphate concentrations, expressed as millimolar concentrations or as mole NTP to mole hemoglobin tetramer, for red cells from pregnant and nonpregnant female, male and fetal rattlesnakes* Crotalus viridis oreganus

Sample	P_{50} at 20°C	P_{50} at 34°C	[NTP] (mmol l^{-1})	NTP/Hb
Pregnant female	6.4±0.8[a]	8.3±0.7[e]	15.5±1.4[i]	3.49±0.33[m]
	N=6	6	5	5
Nonpregnant female	4.9±0.7[b]	8.2±0.8[f]	10.2±0.7[j]	2.41±0.08[n]
	6	6	6	6
Male	4.8±1.0[c]	7.8±0.5[g]	9.8±0.5[k]	2.27±0.58[o]
	6	6	6	6
Fetus	3.4±1.0[d]	6.8±1.4[h]	9.6±2.0[l]	2.47±0.58[p]
	5	4	5	5

Values are expressed as the mean±S.D., *N* is the number of samples for each group, fetal samples represent pooled blood samples from a single litter.

Significant differences in P_{50} data at 20 and 34°C, as indicated by the Tukey test. Single-factor ANOVA was significant at each temperature (F=10.7062 for P_{50} at 20°C, F=3.1424 for P_{50} at 34°C).

$P \leq 0.01$, a:b, a:c, a:d; $P \leq 0.05$, b:d, e:h, f:h; NS, b:c, c:d, e:f, e:g, f:g, g:h.

Significant differences in NTP and NTP/Hb data, as indicated by the Tukey test. Single-factor ANOVA was significant for each variable (F=28.3382 for [NTP], F=14.5327 for NTP/Hb).

$P \leq 0.01$, i:j, i:k, i:l, m:n, m:o, m:p; NS, j:k, j:l, k:l, n:o, n:p, o:p.

NS, not significant.

These results are consistent with the finding of a higher oxygen affinity in fetal red cells than in maternal red cells in many species (see review by Metcalfe *et al.* 1972). Furthermore, fetal rattlesnake red cells had significantly lower NTP concentrations compared with values for maternal red cells (Table 1). Therefore it appears likely, as in other viviparous snakes (Birchard *et al.* 1984; Berner and Ingermann, 1988), that a difference in NTP concentration between fetal and maternal red cells is responsible, at least in part, for the maternal–fetal difference in red cell oxygen-affinity.

At 20 and 34°C the red cell oxygen-affinities for fetal rattlesnakes were significantly greater than those of red cells of the nonpregnant female. Differences between red cell oxygen-affinities for the fetus and male (20 and 34°C) were not significant using the Tukey test. Surprisingly, red cell NTP levels, expressed either as a molar concentration or as a molar ratio of NTP to hemoglobin tetramer, of fetal rattlesnakes were not different from values of either of these adult groups (Table 1). These findings suggest that differences in red cell NTP levels cannot, alone, account for fetal–adult differences in red cell oxygen-affinity.

Nonetheless, red cells from pregnant rattlesnake had a significantly higher NTP level and a significantly lower oxygen affinity, at 20°C, compared to cells from nonpregnant females (Table 1). This shift in red cell oxygen-affinity of the adult may facilitate delivery of oxygen to the developing fetus. These findings of a pregnancy effect in the rattlesnake are similar to the findings in the garter snake (Ingermann *et al.* 1991) and the preliminary findings in the viviparous Australian blacksnake *Pseudechis porphyriacus* (Holland *et al.* 1990).

An increase in temperature generally decreases the oxygen affinity of the red cell of reptiles (Pough, 1969; Greenwald, 1971; MacMahon and Hamer, 1975). Indeed, an increase of 14°C decreased the oxygen affinity of rattlesnake red cells for all experimental groups (Table 1). At 34°C, the red cell oxygen-affinities of adult female rattlesnakes were significantly lower than fetal values; however, there was no difference in red cell oxygen-affinities among the adult groups (Table 1). Thus, pregnancy in this rattlesnake was associated with a significant change in oxygen affinity of red cells from the adult at 20°C, but not at 34°C. These findings are unexpected, compared with the results for the garter snake (Ingermann *et al.* 1991).

The presence of changes in oxygen affinity of adult red cells during pregnancy in mammals is controversial at best. No change in the blood oxygen affinity due to pregnancy was found in studies of humans by Prystowsky *et al.* (1959) and Lucius *et al.* (1970). In contrast, a slight decrease was noted in guinea pigs, cows, goats and humans in several studies (e.g. Hellegers *et al.* 1959; Gahlenbeck *et al.* 1968; Bauer *et al.* 1969; Merlet-Benichou *et al.* 1975). However, the maximum difference in oxygen affinity in pregnant and nonpregnant females, in any of these species, was less than 0.40 kPa (1 kPa=7.50 mmHg). This magnitude of change probably led Battaglia and Meschia (1986) to conclude that pregnancy does not alter the blood oxygen-affinity of the adult to a physiologically significant degree. Results from our laboratory for the garter snake *T. elegans* (Ingermann *et al.* 1991) and rattlesnake *C. v. oreganus* (this study) and the preliminary results of Holland *et al.* (1990) on the blacksnake *P. porphyriacus* indicate that pregnancy is associated with a significant decrease in blood oxygen-affinity of the adult in several snakes. Furthermore, these results, from three representatives of three different families (Colubridae, Viperidae, Elapidae) from two continents, suggest that such an effect of pregnancy may be a widely used physiological strategy in viviparous snakes and possibly other squamates to facilitate maternal–fetal oxygen transfer.

We are grateful to Wendy Bulman for technical assistance and to Mike Mahan for providing rattlesnakes. We are also grateful to Dr Richard Wallace for help in distinguishing pregnant from nonpregnant snakes, as well as for providing animals, and to Dr D. Everson for help with the statistics. This study was supported in part by the Research Council of University of Idaho and in part by grant HD-22391 from the NICHHD, NIH.

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