The Journal of Experimental Biology 202, 2787–2795 (1999)
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JEB1910

EFFECT OF HIGH ALTITUDE AND *IN VIVO* ADENOSINE/β-ADRENERGIC RECEPTOR BLOCKADE ON ATP AND 2,3BPG CONCENTRATIONS IN RED BLOOD CELLS OF AVIAN EMBRYOS

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Accepted 28 July; published on WWW 30 September 1999

Summary

In chick embryos, developmental changes of the blood oxygen tension control hemoglobin (Hb) oxygen affinity *via* modulation of ATP and 2,3BPG concentrations in red blood cells. Hypoxia, which is a normal developmental condition for late chick embryos, causes a decrease of the red cell ATP concentration (and increase of red cell oxygen affinity) as well as activation of 2,3BPG synthesis *via* cyclic AMP-dependent signaling. Adenosine and catecholamines have been implicated as signaling substances in these red cell responses. To assess the extent to which adenosine and catecholamines are involved *in vivo* in the control of red cell ATP/2,3BPG concentrations, day 13 chick embryos were treated for 24 h with adenosine A_2 and/or β-adrenergic receptor blockers and red cell ATP and 2,3BPG levels were determined. The data suggest that adaptive effects later in development in chick embryos induced by adenosine and catecholamines are vital. We have also tested whether avian embryos of the free-living, high-altitude, native white-tailed ptarmigan (*Lagopus leucurus*) alter their organic phosphate pattern in red cells in response to incubation at different altitudes. Embryos incubated at 3600–4100 m decrease their red cell ATP concentration much more rapidly than embryos of the same clutch incubated at 1600 m. From these data it can be inferred that the oxygen affinity of high altitude embryos will be adjusted to the altitude at which the eggs are incubated.

Key words: oxygen tension, hypoxia, ATP, 2,3bisphosphoglycerate, chick embryo, white-tailed ptarmigan, *Lagopus leucurus.*

Introduction

As body mass of avian embryos increases during development toward hatching, embryonic O_2 consumption increases many-fold (Rahn et al., 1974). This progressively larger requirement for O_2 during development is met by increasing hemoglobin concentrations, hematocrits, oxygen diffusing capacity, blood volume, blood flow and affinity of hemoglobin (see review by Tazawa, 1987). At least some of these changes are stimulated by variations in the P_{O_2} inside the shell. Since the O_2 consumption of the embryo increases during development while the conductance of the shell to gas diffusion remains constant (Rahn et al., 1974), the P_{O_2} in the chorioallantoic vein carrying arterialized blood falls continouously throughout incubation from about 90 to 50 mmHg (12.0 to 6.7 kPa) in embryonic domestic chickens (*Gallus domesticus*, Tazawa 1980). The drop in blood P_{O_2} stimulates the synthesis of 2,3bisphosphoglycerate (2,3BPG) and carbonic anhydrase (CAII) and causes the decrease of adenosine triphosphate (ATP) concentration in embryonic red blood cells (RBC) (Baumann et al., 1983, 1986; Ingermann et al., 1983; Million et al., 1991). The changes in ATP and 2,3BPG levels result in an increase in affinity of hemoglobin for O_2 (cf. Isaacks and Harkness, 1980).

In vitro stimulation of CAII and 2,3BPG can be induced by activation of the red cell adenylyl cyclase system *via* adenosine A_{2a}-receptor agonists and β-adrenergic receptor agonists (Dragon et al., 1996; Glombitza et al., 1996). In chick embryos exposed to short-term hypoxia, plasma noradrenaline (NE) concentration is elevated. It is likely that hypoxia is the physiological stimulus for the increase of the NE concentration during normal incubation from day 13/14 onwards (Dragon et al., 1996). However, we do not know to what extent adenosine and NE participate under *in vivo* conditions in the regulation of red blood cell properties.

The finding that oxygen pressure adjusts red cell phosphate pattern and oxygen affinity of chick embryos raises the question of whether the same phenomenon might be operative in the phenotypic adaptation of free-living montane embryos. At least 27 species of birds are known to

breed successfully at altitudes between 4000 m and 6550 m. Several studies on physiological properties of their embryos exist (Carey and Morton, 1976; Rahn 1977; Black and Tenney, 1980; Carey et al., 1982, 1991, 1993, 1994; Carey and Martin, 1997). The mechanisms that contribute to the blood oxygen transport properties of embryos of montane species are not completely understood. In several montane species specific amino acid substitutions cause a small increase of the oxygen affinity of adult hemoglobin (bar-headed geese, *Anser indicus* and Andean geese, *Chloephaga melanoptera*). Inositol pentaphosphate and other allosteric effectors amplify this effect substantially (Petschow et al., 1977; Hiebl et al., 1987; Jessen et al., 1991). During avian embryonic development adult hemoglobins first appear in the definitive red cell line by day 6 in the chick. These cells contain adult hemoglobin and subsequently replace the primitive red cells containing the specific embryonic hemoglobins (Bruns and Ingram, 1973). Initially definitive red cells contain predominantly the high-affinity component HbD in excess of HbA. HbA has a lower intrinsic oxygen affinity. However, despite the fact that the hemoglobin composition of the circulating blood is completely altered during the middle third of incubation, changes in blood oxgen affinity are not closely correlated with altered hemoglobin pattern but instead seem to depend primarily on the red cell organic phosphate composition (Tazawa 1987; Baumann and Meuer, 1992).

Very little information has been obtained on the oxygen-binding characteristics of avian embryos from high altitude species in natural conditions. Snyder et al. (1982) investigated the red cell oxygen affinity and 2,3BPG concentration of montane and lowland embryos (chick, bar-headed geese and Canada geese, *Branta canadensis*). All embryos were incubated at the same altitude of 1700 m. Snyder et al. (1982) could not detect differences in the red cell 2,3BPG concentration when normalizing data to corresponding stages of incubation but, as expected, the oxygen affinity of blood from late embryos of bar-headed geese was higher than that of Canada geese embryos, since at that stage of development the adult hemoglobin pattern is established.

Ptarmigans breed in arctic and alpine tundra habitats that range in elevation from 750 m to 4250 m (Martin et al., 1993). In the present investigation we have analyzed the developmental changes of the red cell phosphate pattern of montane white-tailed ptarmigan embryos (*Lagopus leucurus*) in Colorado. The eggs were incubated at two different altitudes to see if the organic phosphate pattern is affected by the different ambient oxygen pressure. In addition the hemoglobin pattern of late embryos of white-tailed ptarmigan was compared with the pattern of embryos of their lowland congener, willow ptarmigan (*Lagopus lagopus*) from Alaska. The results show that high altitude hypoxia causes changes of the erythrocyte organic phosphate pattern that will increase the oxygen affinity of the montane embryos.

In order to determine the relative importance of β-adrenergic and adenosine receptor activation for the control of red cell ATP/2,3BPG concentration, we investigated whether in vivo blockade of β-adrenergic and adenosine A_2 receptors of late (day 13) chick embryos influences red cell 2,3BPG and ATP levels. Combined application of moderate doses of β-adrenergic and A_2 receptor blocker induces substantial mortality of the chick embryos, which may in part be due to their failure to decrease red cell ATP and increase red cell oxygen affinity, indicating an important role of this hormonal effector system during normal embryonic development.

Materials and methods

Egg collection and incubation

White-tailed ptarmigan eggs were collected from nests located between 3600 m and 4100 m altitude in the alpine tundra of the Rocky Mountains near Mount Evans and Guanella Pass (39 °34–40′ N, 105 °35–53′ W), Clear Creek County, Colorado (Braun et al., 1993). The eggs were left in the nest to be incubated naturally until needed for experimentation. Then, they were collected and transported in a warm insulated box directly to a laboratory at 4270 m near the top of Mount Evans. Since eggs experienced some cooling during transport, they were warmed in a Brower incubator at 36 °C for at least 2 hours before measurements were made.

Another group of white-tailed ptarmigan eggs were collected between 3600 m and 4100 m as soon as the nest was located and were taken to the University of Colorado, Boulder, at 1600 m. They were incubated for a minimum of 9 days at 36 °C, 60 % relative humidity before measurements were made. Several observations led to our assumption that 1600 m is an altitude that is physiologically more similar to sea level than to high altitude for these avian embryos: activities of citrate synthase in muscle and heart of white-tailed ptarmigan embryos incubated at 1600 m differed significantly from those incubated between 3600–4100 m but not from those of willow ptarmigan (*Lagopus lagopus*) embryos incubated at 750 m (C. Carey, J. F. May and K. Martin, unpublished data). Furthermore, no significant differences existed in hematological parameters of turkey (*Meleagris gallopavo*) embryos incubated at 1707 m compared with those of embryos incubated with supplemental O_2 (Bagley et al., 1990).

Blood sampling

Individual eggs were removed from the incubator and the blunt end of the shell was opened. The shell membranes were removed in a manner that protected the chorioallantoic vessels from rupturing. Then the chorioallantoic artery was cut and blood was quickly aspirated.

Embryonic mass

Following blood sampling, the embryos were killed by

cervical dislocation and removed from the egg. The embryo was blotted dry, the yolk sac was removed, and the embryo was weighed on a battery-powered Acculab top-loading balance, accurate to 0.01 g. Red cell ATP and 2,3BPG levels (in mol mol^{-1} Hb) of white-tailed ptarmigan embryos are plotted as a function of embryonic mass because most ptarmigan clutches were found after the onset of incubation and accurate aging was not possible. Furthermore, attempts to estimate incubation age by a flotation method were judged to be inaccurate, because ptarmigan females do not always sit consistently on the eggs after the laying of the terminal egg (Wiebe and Martin, 1995). Masses of white-tailed ptarmigan embryos incubated naturally at 3600–4100 m averaged 11.23 g (N=4, range 10.35–12.21 g) and at 1600 m 11.72 g (N=15, range 10.65–13.62 g) at pipping.

Molar ratios of red cell ATP and 2,3BPG levels

Blood was aspirated with a Pasteur pipette and immediately transferred into ten volumes of ice-cold washing buffer [120 mmol l^{-1} NaCl, 4 mmol l^{-1} KCl, 5 mmol l^{-1} glucose, 1.5 mmol l^{-1} $CaCl_2$, 50 mmol l^{-1} Tris (hydroxymethyl) aminomethane (Tris)], pH 7.4 at room temperature, with 2 i.u. ml^{-1} heparin as anticoagulant). Within 2 min of transfer, the red blood cells were washed three times in washing buffer followed by a short centrifugation (2 s at 13 000 ***g***). A sample (25 µl) of the cell pellet was precipitated using 150 µl 8 % TCA (w/v) at 0 °C. After 5 min the precipitate was pelleted by centrifugation for 5 min at 13 000 ***g***. ATP and 2,3BPG were measured using test kits (Sigma Chemical, Deisenhofen, Germany). The hemoglobin concentration was determined photometrically by the cyan–methemoglobin method.

Hemoglobin pattern

The electrophoretic pattern of hemoglobin was compared between white-tailed ptarmigan (*Lagopus leucurus*) embryos and their lowland congener, willow ptarmigan (*Lagopus lagopus*). Willow ptarmigan eggs were collected within 2 km of the Toolik Field station of the University of Alaska. The station is located north of the Brooks Range at 720 m altitude, 68 °38′ N, 149 °38′ W, in Alaska. To avoid predation, eggs were collected as soon as nests were located and were incubated at the station in a Brower portable incubator at 36 °C and 55 % relative humidity until they were used for measurements. Eggs were rotated 3–4 times per day.

The electrophoretic mobility of hemoglobin from 10–11 g white-tailed ptarmigan and willow ptarmigan embryos was evaluated by PAGE using a discontinuous Tris-glycine buffer system (Snyder, 1978). Sampled blood was washed three times in in 0.85 g % NaCl and the cells were lysed by suspension in distilled water for 20 min. The ghosts were removed by addition of toluene followed by centrifugation at high speed for 30 min. The hemoglobin solution was mixed 1:1 with 12 % sucrose in 0.1 mol l^{-1} Tris buffer, pH 7.4. A sample (30 µl) was applied to a 0.18 % bis-acrylamide stacking gel and run at 75 V. After penetration of the stacking gel, samples were run for 1.5 h at 250 V through a 4 % acrylamide gel. The optical density was scanned at 485 nm with a linear transport module connected to a Gilford Model 250 spectrophotometer and Gilford Model 6051 chart recorder.

Application of adenosine A_2 and β-adrenergic receptor blockers to chick embryos

Since the induction of 2,3BPG synthesis begins around day 13/14 during normal chick embryo development (Isaacks and Harkness, 1975), adenosine and β-adrenergic receptor antagonists were applied as follows. Chick embryos were incubated for 11 days at 37.5 °C and 60 % relative humidity in Regensburg at 400 m. At day 11 a small hole (about 1 mm diameter) was drilled into the blunt end of the egg and immediately sealed with wax. The eggs were then incubated until day 13. At day 13 the seal was removed and adenosine and/or β-adrenergic receptor antagonist dissolved in 200 µl 0.9 g % NaCl was injected onto the inner shell membrane. Control embryos received 200 µl 0.9 g % NaCl solution.

3,7-dimethyl-1-propargylxanthine (DMPX) was used as the adenosine A_2 receptor blocker and propranolol as the β-adrenergic receptor blocker. 6.5 nmol propranolol and/or 0.65 µmol DMPX were injected per egg, which corresponds to about 0.1 µmol propranolol and 10 µmol DMPX kg^{-1} egg mass. After 24 h of further incubation blood was sampled from treated and control eggs and the levels of red cell 2,3BPG and ATP analyzed.

Tests for significant differences ($P \leqslant 0.05$) were carried out with the non-parametric ranking test of Mann and Whitney.

Chemicals

Analytical grade chemicals were purchased from Sigma Chemicals (Deisenhofen, Germany). DMPX and propranolol were obtained from RBI Biotrend (Köln, Germany).

Results

Organic phosphate pattern of white-tailed ptarmigan incubated at 1600 m and 3600–4100 m

The ATP level in RBC of ptarmigan embryos incubated at their normal altitudes (3600–4100 m) was very similar to those of ptarmigan embryos incubated at 1600 m for the youngest ptarmigan embryos weighing about 0.3–0.5 g (Fig. 1A). Highest values were 4.2 mol ATP mol^{-1} Hb. These results are similar to those found in RBC of early chick embryos (Baumann et al., 1983). In embryos of both groups (i.e. high altitude and 1600 m) ATP declined to about 1 mol ATP mol^{-1} Hb in 8 g embryos (Fig. 1A). However, the decrease in ATP was accelerated in high altitude embryos, with the result that embryos weighing 2–6 g had appreciably lower ratios of ATP/Hb than those incubated at 1600 m (Fig. 1A). At a given embryonic mass between

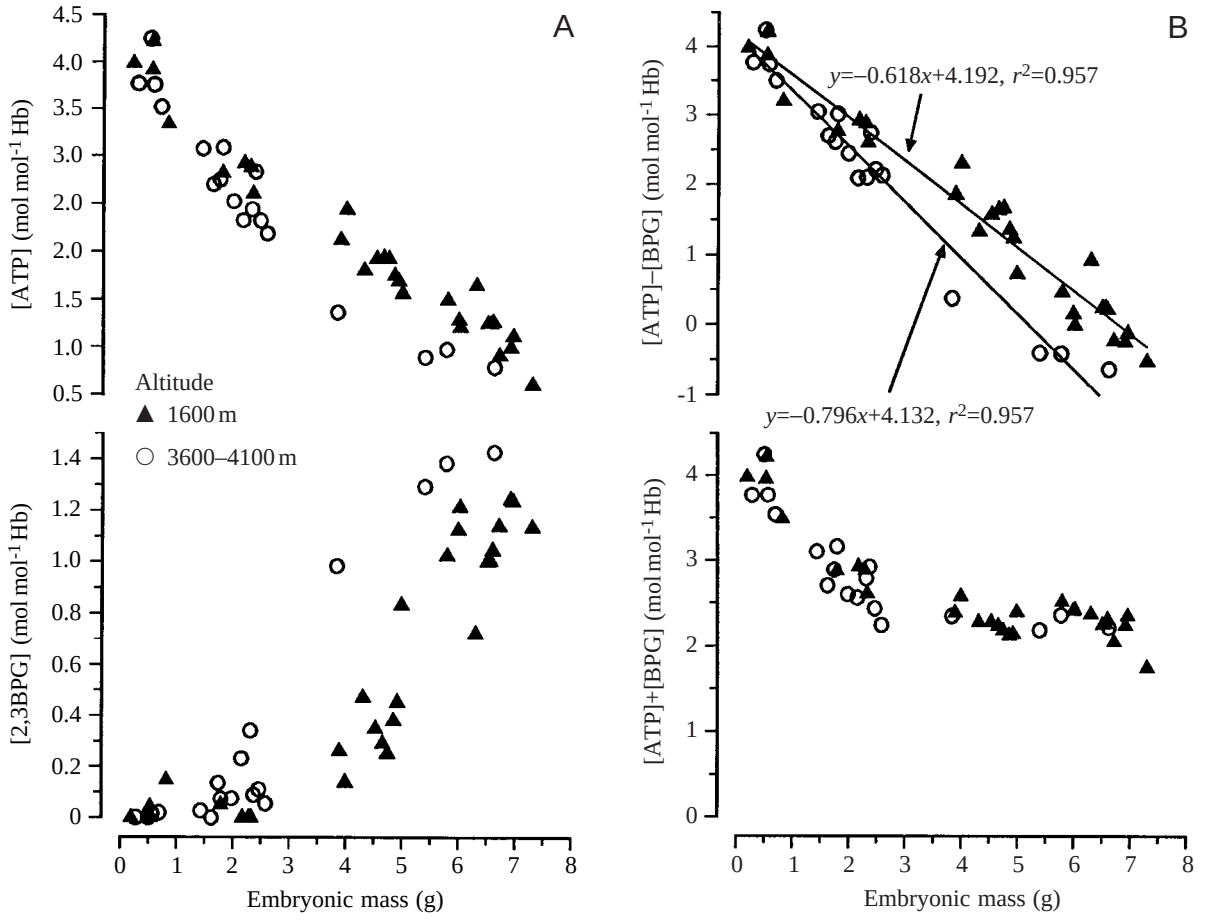

Fig. 1. (A) ATP and 2,3bisphosphoglycerate (2,3BPG) levels (mol mol^{-1} Hb) as a function of embryonic mass (g) in red blood cells of white-tailed ptarmigan embryos incubated at 3600–4100 m (open circles) or transferred for at least 9 days to 1600 m (closed triangles). One embryo provided one ATP value and one 2,3BPG value. (B) Data from A were used to calculate the sum of ATP and 2,3BPG ([ATP] +[2,3BPG], lower panel), and the difference between ATP and 2,3BPG ([ATP]–[2,3BPG]) in red blood cells of individual eggs (upper panel). The two slopes of the linear regression in the upper panel differ significantly (95 % confidence intervals: –0.671 to –0.565, low altitude; –0.886 to –0.706, high altitude). Hb, hemoglobin.

2 and 6 g, maximal differences amounted to about 1 mol ATP mol^{-1} Hb.

The values for 2,3BPG in RBC of 0.5–1.5 g embryos were similar in both groups. 2,3BPG began to increase at a lower embryonic mass (about 2 g) in embryos incubated at 3600–4100 m than in the group incubated at 1600 m and continued to exceed those of the 1600 m group throughout development to a mass of 7–8 g (Fig. 1A).

To allow a better comparison of the two groups and to express the inverse relation of ATP and 2,3BPG levels, the original data from Fig. 1A were replotted in two ways. Because we determined ATP and 2,3BPG together in each sample, we calculated the sum [ATP]+[2,3BPG] for both groups (Fig. 1B). While the total amount of the organic phosphates declines with age, there is no difference between both experimental groups. However, if one assesses the distribution of the organic phosphates between ATP and 2,3BPG by calculating [ATP]–[2,3BPG] for each sample there is a marked difference between the two groups. The linear regression analysis gave in both cases an excellent fit with r^2=0.957 and a significant difference between the two slopes (Fig. 1B).

Hemoglobin pattern

Electrophoretic mobilities of hemoglobins of late white-tailed ptarmigan embryos incubated between 3600 m and 4100 m are virtually identical to those of willow ptarmigan embryos incubated at 720 m (Fig. 2). Two hemoglobin bands, corresponding to adult hemoglobins A and D, were evident in RBC of 10–11 g embryos of both species (Fig. 2).

Red cell ATP and 2,3BPG levels of chick embryos treated with DMPX and propranolol

Fig. 3A shows the distribution of values for erythrocyte 2,3BPG and ATP of 14-day-old chick embryos after treatment with 10 μmol kg^{-1} egg mass of adenosine receptor antagonist DMPX (N=19) for 24 h in comparison to controls (N=18). We classified the molar ratios into categories: in the control group,

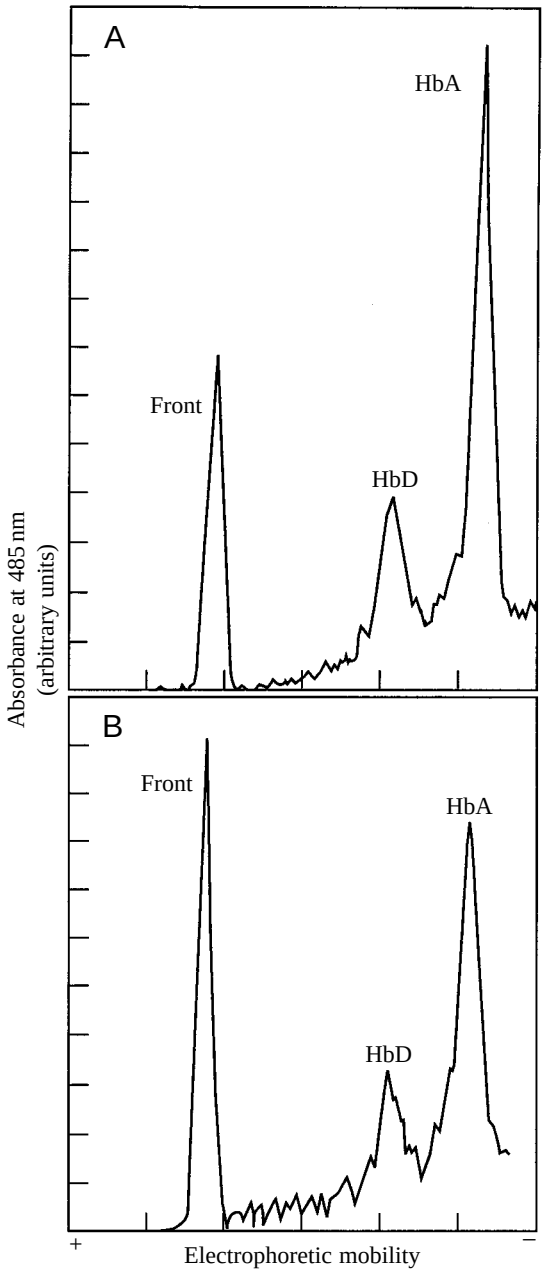

Fig. 2. Densitometric analysis of polyacrylamide disc gel electrophoresis of hemoglobins of late embryonic willow ptarmigan (A) and white-tailed ptarmigan (B). Absorbance was measured at 485 m. Front, the bromophenol blue trace.

47 % had ATP values below 1.4 mol mol^{-1} Hb compared to only 11 % in the group treated with DMPX (Fig. 3B). Similarly, less than 6 % of the control group had a 2,3BPG value <0.4 mol mol^{-1} Hb compared to 33 % in the DMPX group (Fig. 3C). There was no mortality in either group. Statistical analysis by the Mann–Whitney test gave a significant difference of the two groups for 2,3BPG (P=0.0258) and just failed the test for significance (P=0.0566) for ATP.

In the second experiment the embryos had been pretreated for 24 h with 100 nmol propranolol kg^{-1} egg mass. The data (Fig. 4A–C) show similar changes as for the DMPX-treated group: a significant difference (P=0.0133) for 2,3BPG but not for ATP (P=0.0811). Again all embryos survived the treatment.

In the final experiment we assessed the combined effect of treatment with propranolol and DMPX at the same concentrations used before. There were significant differences for ATP and 2,3BPG between the treated group and the control (P=0.0014 for ATP; P=0.0116 for 2,3BPG). In the treated group 25 % (5 of 20) of the embryos died during the 24 h post-injection period. The results for ATP and 2,3BPG (Fig. 5A–C) show extensive differences in the distribution of ATP and 2,3BPG values. In the control group more than 63 % of the embryos had ratios for red cell 2,3BPG/Hb of >0.6 mol mol^{-1} Hb and 68 % had for ATP/Hb of <1.6 mol mol^{-1} Hb (compared to 27 % of the treated group). 53 % of the embryos treated with DMPX and propranolol had ratios for 2,3BPG/Hb of <0.4 mol mol^{-1} Hb and for ATP/Hb of >1.8 mol mol^{-1} Hb, compared to only 11 % of the control group.

In conclusion the combined application of moderate doses of adenosine and B-adrenergic receptor blockers largely inhibits the decline in ATP and the increase in 2,3BPG levels in red cells. Taken together the results support the idea that both adenosine and noradrenaline mediate the switch in organic phosphates due to the obligatory developmental hypoxia in chick embryos.

Discussion

Hypoxia modulates the red cell phosphate pattern of montane avian embryos

The normal developmental changes of ATP and 2,3BPG levels in RBC of chick and other avian embryos cause an increase in the oxygen affinity of the hemoglobins (cf. Isaacks and Harkness, 1980). In embryonic white-tailed ptarmigan the developmental changes of organic phosphates in RBC follows the pattern established for chick embryos (Mission and Freeman, 1972; Isaacks and Harkness, 1975; Bartlett and Borgese, 1976; Baumann et al., 1983; Ingermann et al., 1983). In addition, the embryos incubated at 3600–4100 m show an accelerated decline of the erythrocyte ATP level during development, compared to that of embryos incubated at 1600 m, which begins prior to the onset of massive weight gain (i.e. embryos that have attained only 10–15 % of their hatching mass). The difference in red cell ATP between both groups is substantial in the mass range 3–6 g. High altitude embryos have up to 1 mol ATP mol^{-1} Hb less than the controls incubated at 1600 m. Correspondingly, we observe an earlier stimulation of 2,3BPG synthesis in the high altitude group. The results are similar to the findings obtained with chick embryos exposed to chronic hypoxia, where differences in ATP and 2,3BPG concentration of normoxic and hypoxic

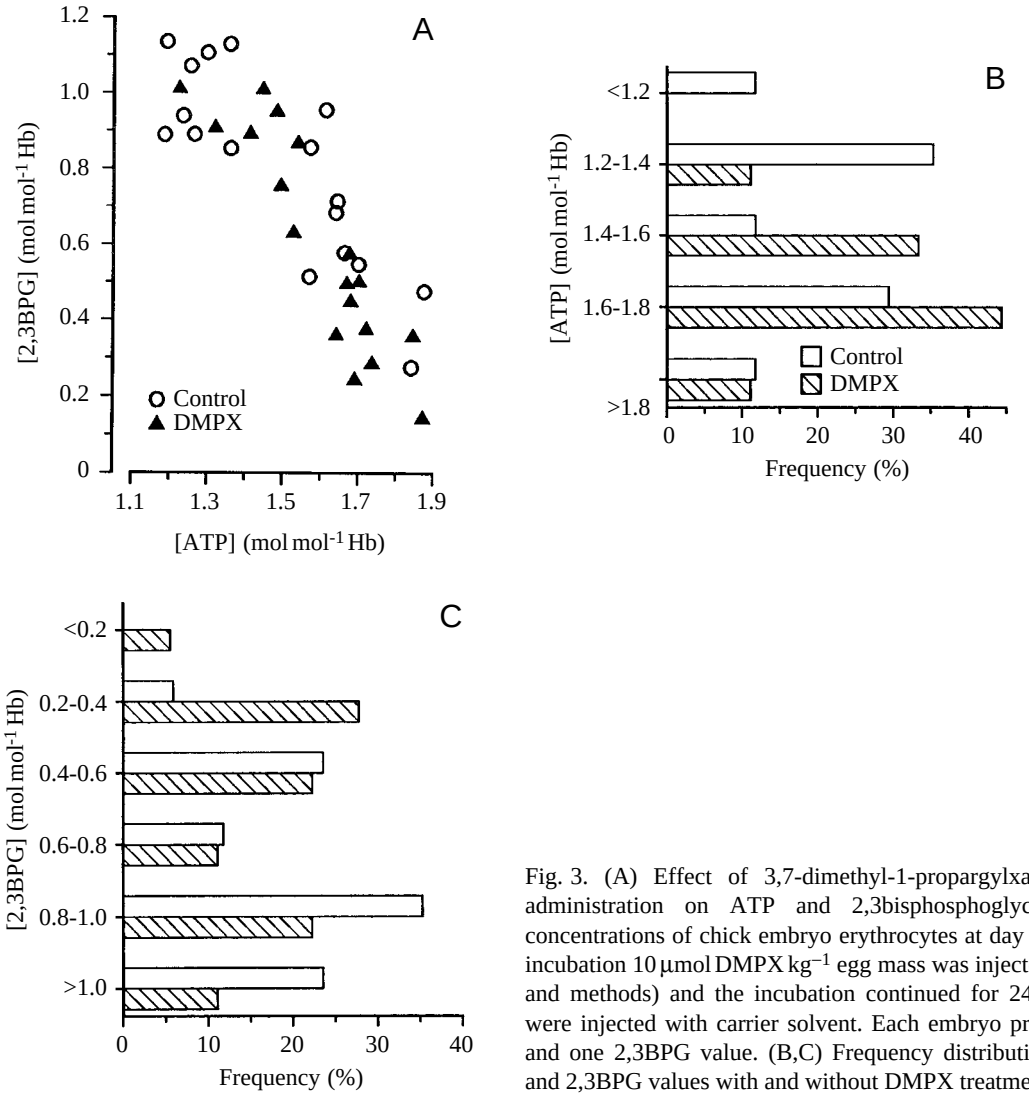

Fig. 3. (A) Effect of 3,7-dimethyl-1-propargylxanthine (DMPX) administration on ATP and 2,3bisphosphoglycerate (2,3BPG) concentrations of chick embryo erythrocytes at day 14. At day 13 of incubation 10 μmol DMPX kg^{-1} egg mass was injected (see Materials and methods) and the incubation continued for 24 h. Control eggs were injected with carrier solvent. Each embryo provided one ATP and one 2,3BPG value. (B,C) Frequency distribution (%) for ATP and 2,3BPG values with and without DMPX treatment.

embryos become apparent after day 7 of incubation (Baumann et al., 1983, 1986). As stated in the Introduction, the circulating blood contains variable amounts of primitive and definitive erythrocytes, depending on the developmental stage. Million et al. (1991) showed that during hypoxia the stimulation of 2,3BPG synthesis in the chick embryo occurs in definitive as well as mature primitive erythrocytes. Therefore, the affinity of both adult and embryonic hemoglobins would be affected. The same phenomenon may be inferred for white-tailed ptarmigan embryos developing at high altitude.

In conclusion, the present results suggest that the same oxygen-tension-dependent mechanism controls the red cell organic phosphate pattern of montane and lowland embryos. This would also explain the data of Snyder et al. (1982), who found that the activation of 2,3BPG synthesis occurs at equivalent developmental stages in chick embryos, Canada geese and bar-headed geese embryos incubated at the same altitude of 1700 m.

Although hemoglobin affinity of white-tailed ptamigan embryos was not determined in this study, we tentatively conclude that, due to the rapid decay of red cell ATP (which is more powerful than 2,3BPG as an allosteric effector of avian hemoglobins; cf. Isaacks and Harkness, 1980), the oxygen affinity of the hemoglobins of embryos incubated at 3600–4100 m will also be increased compared to the siblings incubated at 1600 m.

The affinity of hemoglobins for oxygen may be altered by a number of other factors, including the amino acid sequence of the hemoglobins. Several species of birds that fly and breed at very high altitudes have amino acid substitutions in the globin chains that affect hemoglobin affinity and their interaction with allosteric effectors (Petschow et al., 1977; Hiebl et al., 1987; Jessen et al., 1991). The electrophoretic mobilities of the hemoglobins A and D of late white-tailed and willow ptarmigan embryos do not differ (Fig. 2), although not all amino acid substitutions are revealed by electrophoresis.

It should be realized that the advantage conferred by

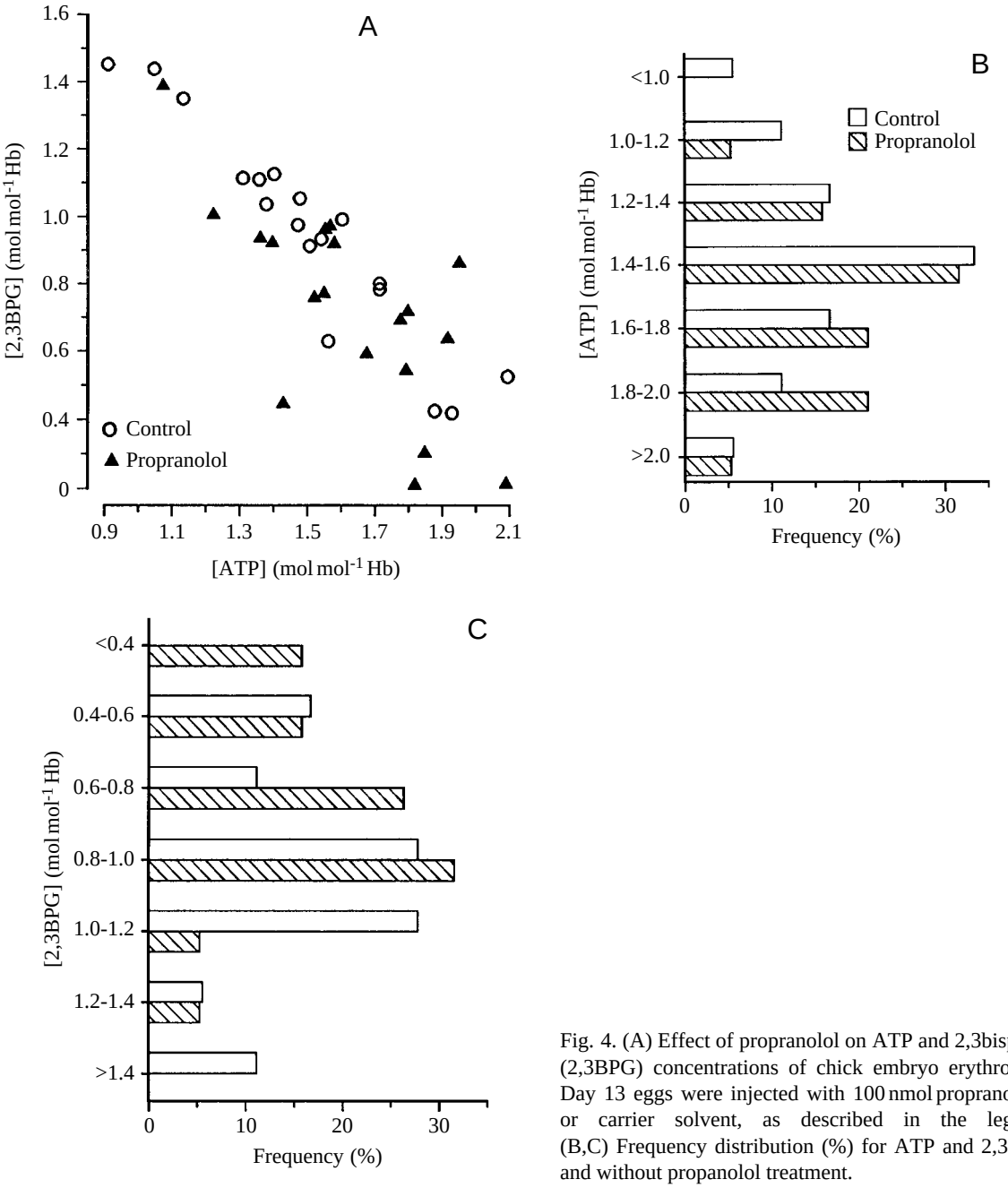

Fig. 4. (A) Effect of propranolol on ATP and 2,3bisphosphoglycerate (2,3BPG) concentrations of chick embryo erythrocytes at day 14. Day 13 eggs were injected with 100 nmol propranolol kg^{-1} egg mass or carrier solvent, as described in the legend to Fig. 3. (B,C) Frequency distribution (%) for ATP and 2,3BPG values with and without propanolol treatment.

possessing adult hemoglobin with a higher intrinsic oxygen affinity can manifest itself only late in development, when the adult hemoglobin pattern is fully established. But for avian species breeding at high altitude, the changes of the embryonic red cell ATP and 2,3BPG pattern induced by hypoxia in early and mid-term development will increase oxygen affinity irrespective of the hemoglobin type.

Role of adenosine and catecholamines in adaptation to hypoxia

Our second set of experiments investigated the extent to which NE and adenosine participate under *in vivo* conditions in the oxygen-pressure-dependent regulation of organic phosphate synthesis. Both substances have been implicated to be involved in the regulation by *in vitro* experiments (Dragon et al., 1996; Glombitza et al., 1996) and we have shown that both developmental and enviromental hypoxia causes release of NE into the embryonic circulation (Dragon et al., 1996). We therefore tested how the application of adenosine A_{2a} receptor blocker or β-adrenergic receptor blocker to day 13 chick embryos influenced the molar red cell ATP and 2,3BPG ratio measured in the same embryos at day 14 in comparison to sham-treated controls. We selected this stage since the stimulation of 2,3BPG synthesis begins at this time during normal development.

As expected, the data for ATP and 2,3BPG of day 14 control chick embryos showed large variations, in keeping with the

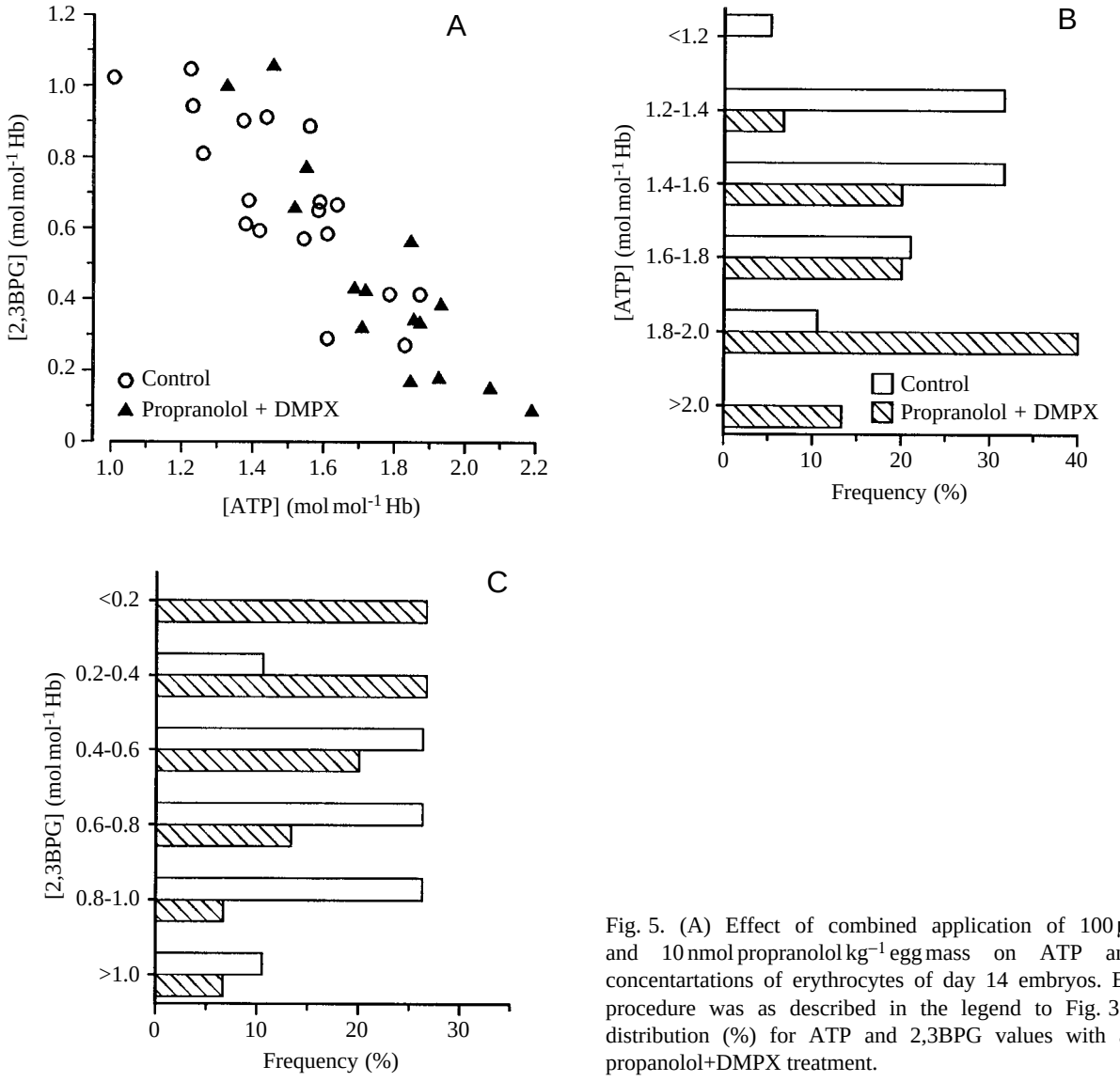

Fig. 5. (A) Effect of combined application of 100 µmol DMPX and 10 nmol propranolol kg^{-1} egg mass on ATP and 2,3BPG concentartations of erythrocytes of day 14 embryos. Experimental procedure was as described in the legend to Fig. 3. Frequency distribution (%) for ATP and 2,3BPG values with and without propanolol+DMPX treatment.

finding that differences in egg shell gas conductivity as well as egg size have a pronounced influence on the timing of hypoxia and hypercapnia (cf. Tazawa, 1987). Differences in chorioallontoic venous oxygen pressure of up to 20 mmHg are encountered at this stage and mirrored in different red cell organic phosphate concentrations (Koller et al., 1994) and large variations of oxygen affinity (Tazawa, 1987).

The single and, in particular, the combined application of β-adrenergic and adenosine A_2-receptor blockers to 13-day-old chick embryos for 24 h significantly inhibits *de novo* 2,3BPG synthesis and stabilizes ATP in the erythrocytes of treated embryos as compared to the controls. Blocking both receptor types therefore precludes the continuous adaptation of the blood oxygen affinity to the falling oxygen tension in the chorioallantois, where under normal conditions the oxygen half-saturation pressure can decrease by more than 10 mmHg within 24 h (Tazawa, 1987). It is noteworthy that the combined application of adenosine and β-adrenergic blockers induced substantial mortality, as 25 % of the treated embryos died within 24 h. The doses for propranolol and DMPX used in our experiments were moderate, which is also reflected by the result that single application had no effect on mortality. We cannot exclude the possibility that other organs besides erythrocytes are adversely affected by the receptor blockade, but the results indicate that the adaptations mediated by adenosine and β-adrenergic receptors are vital for further embryonic development in the last part of the incubation period.

Support by DFG grant (Ba 691/5–1) is gratefully acknowledged.

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