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Hematologic and Body Fluid Changes during Simulated High Altitude Exposure in Naproxen-Treated Rats

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Abstract: Arterial blood oxygen saturation, body fluid and hematological parameters were studied in control, naproxen (a prostaglandin synthesis inhibitor)-treated control, altitude-exposed, and naproxen-treated altitude-exposed rats after intermittent exposure of 8 h/d for 6 consecutive days to a simulated high altitude of 6,100 m (barometric pressure 349±3 mmHg). Arterial blood oxygen saturation was reduced in altitude-exposed rats, but increased significantly to near control level in naproxen-treated rats. On the other hand, 2,3-diphosphoglycerate in erythrocytes increased in altitude-exposed rats, but naproxen prevented this increase. The red blood cell count, hemoglobin concentration and hematocrit ratio were reduced significantly in drug-treated altitude-exposed rats when compared to the altitude-exposed group (without drug). Red cell mass was increased in the altitude-exposed group in comparison with control. Both red cell mass and mean corpuscular volume of altitude-exposed rats were shifted towards the control value when they were treated with naproxen. Drug-treated high altitude-exposure reduced the thiocyanate space, intracellular fluid volume and total body water content compared to drug-treated control rats. On the other hand, thiocyanate space and total body water content were increased significantly in drug-treated control rats in comparison with the control group. [Japanese Journal of Physiology, 46, 67–73, 1996]

Key words: hypoxia, oxygen saturation, hematocrit.

The physiological responses induced by hypoxia have been well studied in mammalian species. An increase in red cell count, hemoglobin concentration and hematocrit [1–3] and a decrease in plasma volume, blood volume and total body water content have been reported [4, 5] during continuous exposure to high altitude. A reduction in arterial oxygen saturation [6] and an increase in 2,3-diphosphoglycerate (2,3-DPG) concentration in the erythrocyte [4] have also been observed.

Hypoxia stimulates the production and release of erythropoietin and progenitor cells and this has been attributed to prostaglandins [7]. High altitude polycythemia provide an adaptive advantage because a greater amount of oxygen can be carried at a given arterial P_{O_2}. Therefore, the successful adaptation of an animal at a high altitude environment may be dependent on prostaglandin (PG). In accord with this idea, Vaughan *et al.* [8] observed a reduction of the high hematocrit value normally induced by simulated high altitude after treatment witn aspirin (a PG synthesis inhibitor). It is known that naproxen and aspirin are effective inhibitors of cyclooxygenase which is responsible for the biosynthesis of prostaglandins, but that there is considerable variation in their potency. Notably, naproxen is approximately 20 times more potent than aspirin [9]. Therefore, we wondered whether further studies with naproxen would uncover the role of prostaglandins in the erythropoietic response in chronic hypoxia. Thus, the present study was undertaken to investigate the effects of PG inhibition by naproxen on the hematological responses and body fluid adjustments induced by chronic hypoxia.

MATERIALS AND METHODS

Sixty-four young male albino rats that usually weighed 120 to 135 g were used in the present study.

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Thirty-two rats were used for the body fluid and hematological study and the other 32 for blood oxygen saturation and red cell 2,3-DPG measurements. The rats were divided into four groups with 8 rats in each group: Group I was maintained at sea level (barometric pressure 760 mmHg). Group II was maintained at sea level (760 mmHg) but treated with naproxen. Group III were exposed to simulated high altitude of 6,100 m (barometric pressure of 349± 3 mmHg). Group IV were exposed to a simulated high altitude of 6,100 m and treated with naproxen.

Sixteen rats in groups III and IV were placed in a decompression chamber which was set up according to the method of Purshottam and Ghosh [10] and Biswas *et al.* [11]. The chamber temperature was maintained at 28±1°C. Simulated high altitude was maintained by adjusting the chamber pressure to 349±3 mmHg. A constant flow of fresh air inside the chamber was maintained throughout the exposure to reduced atmospheric pressure and therefore hypoxia was intermittent, limited to 8 h/d for 6 consecutive days. The rate of compression and decompression was adjusted to 610 m (53 mmHg)/min.

The rats in groups II and IV were treated with naproxen (12 mg/kg/d). The daily dose of naproxen (suspension in 10% alcohol) was divided equally into two parts; one part was given orally through a gastric cannula attached to 1-ml syringe before and the other part after the hypoxic exposure on each day. Rats in groups I and III received the same amount of 10% alcohol alone. The rats were fed standard laboratory food and tap water *ad libitum*. All the measurements were performed on the rats when they were breathing room air (immediately after the 6th day of exposure). On the 6th day, 32 rats (groups I, II, III and IV) were anaesthetised with ether and 0.2 ml of a solution containing equal volumes of 5% sodium thiocyanate and 0.5% Evan's blue (T-1824) was injected into the surgically exposed femoral vein from a syringe through a needle which was weighed pre- and post-injection [12]. One minute later, the femoral vein was ligated to the site of injection. About 0.7 ml of blood was collected from the vena cava via a cannula exactly 2, 4 and 8 min after the injection into a syringe containing a small amount of heparin to prevent coagulation. Light ether anesthesia was continued throughout the procedure. Heparin 10 U/ml of saline was used to prevent coagulation within the cannula.

Measurement of body fluid and hematological data. Measurement of plasma volume, thiocyanate space (extracellular fluid volume), total body water and calculated intracellular fluid volume were carried out as described previlusly [11, 13]. The rate of disappearance of Evan's blue and of sodium thiocyanate were measured in the plasma samples from each animal and was plotted on semilogarithmic graph paper. Plasma volumes and extracellular fluid volumes of all groups of rats were calculated from the previously constructed semilogarithmic decay plots of Evan's blue and sodium thiocyanate as described in our earlier works [11, 13]: the zero-time value was extrapolated from the Evan's blue and sodium thiocyanate concentrations of plasma found in 2, 4 and 8 min post-injection samples of each animal.

Blood volume was calculated from the plasma volume and hematocrit using Loring's formula [14]. Thiocyanate space was estimated according to the method of Wang and Hegsted [15]. Total body water was calculated from the initial body weight and the final dry body weight after desiccation for 48 h at 80°C according to the previous work from this laboratory [11, 13, 16]. Intracellular fluid volume was calculated from the difference between total body water and thiocyanate space. The blood samples taken from these rats were also used to count red blood cells and to measure the hemoglobin concentration and hematocrit value described previously [16]. Red cell mass, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from these results.

Measurement of blood oxygen saturation. Eight rats in each of the four groups (total of 32 rats) were used for the determination of blood oxygen saturation and for the measurement of red cell 2,3-diphosphoglycerate (2,3-DPG). On the 6th day, immediately after the exposure to hypoxia, blood was carefully drawn from the dorsal aorta of the ether-anaesthetised rat directly in a suitably greased and heparinised syringe via a needle to avoid contact with air. The blood was transferred to a 2-ml syringe containing freshly prepared saturated saponin solution in its dead space (about 0.07 ml). After hemolysis, blood was transferred to a spectrophotometric cuvette and oxygen saturation was measured according to the method of Falholt [17]. The P_{O_2} value in the room air was 152±2.0 during the measurement of blood oxygen saturation. Air contact was avoided throughout the procedure.

Estimation of red cell 2,3-DPG. Red cell 2,3-DPG was measured by a colourimetric method [18]. It was measured in protein-free supernatant after centrifuging the blood with ice-cold trichloracetic acid in a cold centrifuge. After reaction of the sample with the enzyme (phosphoglycerate mutase), the liberated inorganic phosphate was measured in a spectrophotometer (660 nm). The concentration of 2,3-DPG in

the sample was calculated by comparison with a standard sample. The 2,3-DPG concentration was expressed in terms of hemoglobin content.

Statistics. For statistical analysis, analysis of variance (ANOVA) and Student's *t*-test were performed in this investigation to evaluate the significance of changes of the experimental data. The results are shown in the tables and figures as the mean±SE.

RESULTS

Body weight

The mean body weight in the control rats (group I) and naproxen-treated control rats (group II) increased from 126.0±1.5 to 135.87±3.07 g and 123.0±1.9 to 124.13±2.69 g, respectively, over the 6 d of the experiment. In contrast, the changes in groups III and IV were 128.87±2.66 to 126.0±2.72 g and 127.5±3.66 to 126.88±2.64 g, respectively. The body weight gain was significantly suppressed in group II rats in comparison with group I and no significant body weight gain was observed in group III and IV rats after 6 d of exposure. The suppression of body weight gain in group III and IV rats were statistically significant when compared to group I rats.

Blood oxygen saturation and red cell 2,3-DPG

Arterial blood oxygen saturation and red cell 2,3-DPG values are shown in Figs.1 and 2. Arterial blood oxygen saturation in group III was significantly lower than that in group I. On the other hand, group IV showed significant improvement in arterial oxygen saturation in comparison with group III. The red cell 2,3-DPG level increased significantly in group III in comparison with group I, but the effect was reduced by naproxen in group IV compared to group III.

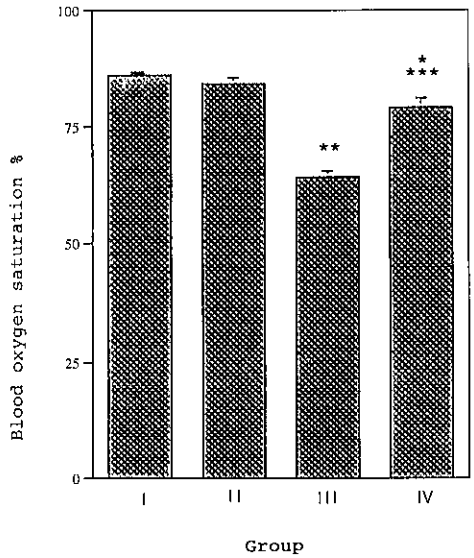

Fig. 1. Blood oxygen saturation. Blood oxygen saturation % of group I (control), II (drug-treated control), III (altitude) and IV (drug-treated altitude) rats. Mean values of 8 rats in each case. Vertical lines represent mean±SE. *F* value and *p* value are of ANOVA. $F=46.27$, $p<0.001$. Student's *t*-test, compared to group I, $*p<0.01$, $**p<0.001$; compared to group III, $***p<0.001$.

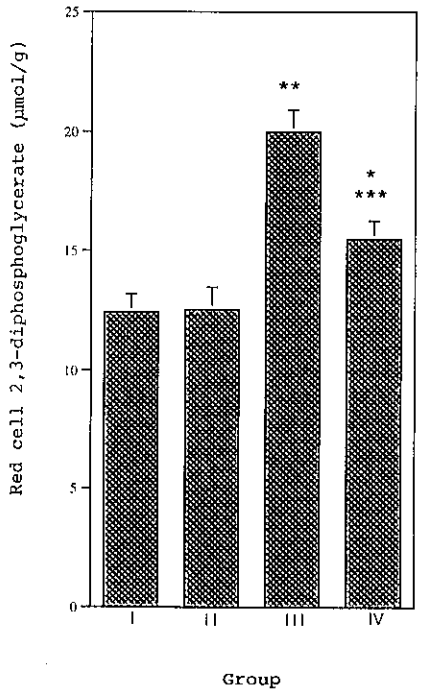

Fig. 2. Red cell 2,3-DPG. Red cell 2,3-DPG μmol/g of hemoglobin of group I (control), II (drug-treated control), III (altitude) and IV (drug-treated altitude) rats. Mean values of 8 rats in eacha case. Vertical lines represent mean±S.E. *F* value and *p* value are of ANOVA. $F=17.35$, $p<0.001$. Student's *t*-test, compared to group I, $*p<0.02$, $**p<0.001$; compared to group III, $***p<0.01$.

Hematological changes

The hematological data are shown in Table 1. Significant increases in red blood cell count, hemoglobin concentration and hematocrit were observed in group III relative to group I. These increased effects of altitude exposure were suppressed significantly in group IV rats after treatment with naproxen. A significant decrease in MCV was observed in group III relative to group I but no such alteration was noticed in group IV in comparison with group I.

Body fluid

The changes in the body fluid parameters are shown in Table 2. A significant increase in red cell mass was observed in group III animals relative to group I; this increase was slightly smaller in group IV compared to group III. Total body water was decreased in group III compared to group I rats. Thiocyanate space, intracel-

Table 1. Hematological parameters of control (group I), drug-treated control (group II), altitude (group III) and drug-treated altitude (group IV)-exposed animals.

	n	Control (I)	Control with drug (II)	Altitude (III)	Altitude with drug (IV)	ANOVA	
						F value	*p* value
RBC	8	6.39±0.135	6.41±0.124	7.7±0.23††	6.91±0.12**,†	14.8	<0.001
Hb	8	16.4±0.27	16.65±0.23	19.15±0.37††	18.03±0.26*,††	19.59	<0.001
Hct	8	45.63±0.86	45.77±0.59	52.26±1.02††	48.93±0.49***,†	16.68	<0.001
MCV	8	71.42±0.31	71.46±0.62	68.06±0.78***	70.96±1.13	5.03	<0.01
MCH	8	25.69±0.28	25.99±0.32	24.95±0.47	26.13±0.36	2.08	N.S.
MCHC	8	35.96±0.25	36.36±0.2	36.67±0.53	36.83±0.26	0.6	N.S.

Values are means±SE, *n*=number of animals in each group. RBC=10^6 red blood cell/mm^3; Hb=hemoglobin g/100 ml blood; Hct=hematocrit %; MCV=mean corpuscular volume, u; MCH=mean corpuscular hemoglobin, pg; MCHC=mean corpuscular hemoglobin concentration, %. N.S.=not significant. Student's *t*-test, compared to group I, $^{**}p<0.02$, $^{***}p<0.01$, $^{\dagger\dagger}p<0.001$; compared to group III, $^{*}p<0.05$, $^{\dagger}p<0.01$.

Table 2. Body fluid parameters of control (group I), drug treated control (group II), altitude (group III) and drug treated altitude (group IV) exposed animals.

	n	Control (I)	Control with drug (II)	Altitude (III)	Altitude with drug (IV)	ANOVA	
						F value	*p* value
Red cell mass	8	3.38±0.18	3.68±0.11	4.25±0.22†	3.99±0.08†	5.71	<0.01
Plasma volume	8	4.1±0.16	4.48±0.123	4.01±0.12	4.33±0.12	2.65	N.S.
Blood volume	8	7.32±0.33	7.99±0.21	8.07±0.3	8.165±0.18	2.2	N.S.
Thiocyanate space	8	12.02±0.38	14.24±0.37††	12.37±0.4	12.94±0.46**	5.83	<0.01
Intracellular fluid volume	8	56.42±0.26	57.77±0.81	54.16±0.48	55.1±0.59***	7.61	<0.01
Total body water	8	68.43±0.61	72.01±0.83†	66.53±0.58*	68.05±0.55†††	12.61	<0.001

Values are means±SE in 100 g body weight, *n*=number of animals in each group. NS=not significant. Student's *t*-test, compared to group I, $^{*}p<0.05$, $^{\dagger}p<0.01$, $^{\dagger\dagger}p<0.001$; compared to group II, $^{**}p<0.05$, $^{***}p<0.02$, $^{\dagger\dagger\dagger}p<0.01$.

lular fluid volume and total body water were reduced significantly in group IV relative to group II animals. On the other hand, thiocyanate space and total body water were increased significantly in group II rats compared to group I.

DISCUSSION

The physiological changes observed in the rats upon intermittent exposure to simulated high altitude are qualitatively similar to those of other mammalian species studied after continuous exposure [1, 3, 19, 20]. The results of the altitude-exposed (group III) and naproxen-treated altitude-exposed (group IV) rats suggested that the acclimatization of the rats is accompanied by suppression of body weight gain. The low weight gain in group III may be partly due to hypo-hydration which is evidenced by the significant reduction of total body water. Similar observations were also observed in our previous studies with toads and rats [11, 21]. Picon-Reategui *et al.* [5] have reported that a 19% reduction in body weight in the rat after hypoxic exposure was due to the loss of water. On the other hand, reduction of body weight gain in group IV rats could not have been due to hypohydration because this group of rats did not show any decrease of total body water. Flower *et al.* [22] have shown that large doses of aspirin (an inhibitor of PG synthesis) cause depletion of liver and muscle glycogen, and also breakdown of proteins. Recently, it has been observed that treatment with naproxen at altitude causes a significant reduction in liver weight in rats [21]. Therefore, the suppression of body weight gain in group IV may have been caused by the metabolic effects of this drug rather than by hypohydration.

In the present study, arterial blood oxygen saturation decreased significantly when rats were exposed intermittently to a simulated high altitude 6,100 m. The cause of this reduced arterial blood oxygen saturation is thought to be the increased level of 2,3-DPG in this group of animals. Similar results have been observed by several investigators [6, 23] after exposure to altitudes of 6,100 to 6,710 m. On the other hand, the exact cause of increase of blood oxygen saturation

in group IV rats is not fully clear. It has been reported that prostaglandins are involved in hypoxic hypoxia, in stimulation of erythropoietin and subsequent recruitment of red cells [7]. Hypoxia promotes prostaglandin synthesis in the kidney with various types of stimuli [24]. It produces ischemia in the kidney, renal nerve stimulation and release of angiotensins from this organ and catecholamines from the adrenal medulla. During the hypoxic condition, mitochondria elaborate increasing amounts of free fatty acids from the ischemic tissues. It is postulated that these free fatty acids and polyunsaturated precursors arachidonic acid are converted into prostaglandins. The prostaglandin endoperoxides generated from arachidonic acid, is released by the cells under hypoxic conditions by a mechanism which ultimately stimulates the release of renin and angiotensin, and which also may be stimulated by catecholamines. Finally, the prostaglandins may lead to increased erythropoietin by the kidney (renal cortex) although extrarenal erythropoietin formation from angiotensin is also reported. Prostaglandins also stimulate erythropoiesis directly at the marrow cell level. Thus, it may be proposed that the release of prostaglandin together with increase in level of red cell 2,3-DPG after hypoxic exposure was responsible for the decrease in blood oxygen saturation in group III animals compared with the control level, and that blood oxygen saturation was improved by treatment with naproxen. The present experiments also showed an increased level of red cell 2,3-DPG after altitude exposure in comparison with the control group. Several investigators [4, 25] have similarly reported that hypoxia at altitude increrases the concentration of 2,3-DPG in red blood cells. It is known that increasd accumulation of 2,3-DPG facilitates its binding with hemoglobin and to decreases the affinity of hemoglobin for oxygen, causing displacement of the oxygen-hemoglobin dissociation curve to the right [26]. In the present study, the reduced level of red cell 2,3-DPG in group IV rats in comparison with group III might therefore explain the increase of blood oxygen saturation in group IV relative to group III.

The increases in the number of blood cells and in hemoglobin and hematocrit in group III rats was thought to be due to hemoconcentrarion following slight decrease in plasma volume and a significant increase in red cell mass relative to group I. Similar increases in these parameters was observed in rats after continuous and intermittent exposures [27, 28]. On the other hand, both plasma volume and red cell mass are slightly increased in group II in comparison with group I. There was a marked reduction in the red blood cell count, hemoglobin concentration and hematocrit ratio in group IV rats compared to group III. This indicated that exposurre to altitude fails to stimulate sufficiently to increase these parameters when rats are treated with naproxen. This inhibition of erythropoietic activity by PG synthesis-inhibiting drugs may be due to the inhibition of erythropoietin production. Fisher *et al.* [7] have shown that the PG synthesis inhibitor indomethacin blocked the production of erythropoietin by the kidney during hypoxic hypoxia. It is tempting to speculate that naproxen may also operate by a similar mechanism. However, this requires confirmation. Several investigators have observed altitude exposure increases in splenic weight in mice and rats [29, 30], and this increase was suggest to be the result of increased hemopoiesis in this organ. In our previous observation [21], the increased splenic weight of the hypoxia-exposed rat was reduced after treatment with naproxen in hypobaric hypoxia and it was thought to be related to the inhibition of erythropoietic activity of this organ. The decrease of calculated MCV in response to altitude exposure is considered to be due to hypohydration of this group of rats and thereby they accomodate lower MCH. Similar results were observed by Feigen and Johnson [27] in rats at natural high altitude. On the other hand, the unaltered MCV in group IV rats may be explained by the unaltered total body water in this group of rats in comparison with the control group. The increase in red cell mass in group III rats indicated an increase in erythropoiesis in this group. The slight decrease in red cell mass in group IV rats may be considered to be due to the reduction of erythropoiesis by naproxen through inhibition of erythropoietin release. The present investigation failed to show any significant alteration in plasma volume and blood volume in group III and IV rats in comparison with group I rats. A slight increase in plasma volume was also noticed in group II and IV animals relative to group I. This indicated that this short-term intermittent high altitude exposure did not affect much on plasma volume and blood volume. On the other hand, thiocyanate space and total body water in group II rats were increased significantly compared to group I, indicating retention of water after naproxen treatment at normal atmospheric pressure.

The increase of total body water in group II rats in comparison with group I showed retention of water after treatment with naproxen at normobaric pressure. The significant reduction of total body water in group III rats in comparison with group I indicated dehydration of this group of rats. A similar dehydration occurred in group IV rats compared to group II rats. On the other hand, the significant increase in total body

water in group IV rats in comparison with group III rats indicated that dehydration did not occur in this group of rats. Therefore, application of naproxen may prevent the loss of total body water in rats when exposed intermittently at simulated high altitude. It has shown that retention of water occurs after treatment with aspirin in certain diseases [22]. From our present investigation, we observe that responses in altitude-exposed rats consisted of body fluid loss which possibly altered hematological parameters through hemoconcentration. The responses of the drug-treated altitude-exposed group showed improvement in body fluid parameters, especially the total body water, relative to group III rats, and this effect was reflected in the hematological parameters. In group IV rats, hemoconcentration was reduced after naproxen treatment in comparison with group III rats.

The present study indicates that the significant alteration of hematological parameters towards the control level in group IV rats may be due to the inhibition of erythropoietin secretion and that this occurs by the reduction of prostaglandin release. Therefore, from this investigation, we may infer that inhibition of hypoxic release of prostaglandins by naproxen may have an important role in the process of acclimatization. The decreased concentration of red cell 2,3-DPG coupled with increased blood oxygen saturation after naproxen treatment may be helpful in this process. The physiological mechanism operating in naproxen-treated and altitude-exposed rats needs further investigation, but this study has identified some of the physiological changes associated with drug treatment and chronic hypoxic exposure in rats.

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