A protective effect of glucocorticoids in hypoxic stress

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Roosevelt, T. Steven, Ann Ruhmann-Wennhold, and Don H. Nelson. A protective effect of glucocorticoids in hypoxic stress. Am. J. Physiol. 223(1): 30-33. 1972.—Studies were carried out in female rats on the effects of glucocorticoids on survival time and lactic acid formation in hypoxic stress. Endogenous corticoids increased survival time of rats exposed to a hypoxic (5% O2) atmosphere, since sham-adrenalectomized animals survived significantly longer than adrenalectomized controls. Administration of cortisol had a similar effect in adrenalectomized rats. The difference in survival times is probably not due to the absence of the adrenal medulla, since sham-hypophysectomized rats survived hypoxia significantly longer than hypophysectomized rats. Blood lactate was elevated in hypoxic animals but, more significantly, intact animals had lower levels of blood lactic acid than adrenalectomized animals. Animals replaced with cortisol following adrenalectomy also had lower levels of blood lactate following exposure to hypoxia than adrenalectomized controls. Corticoids probably protect against hypoxia through a facilitation of aerobic metabolism.

Blood lactic acid; hypoxia; hypophysectomy

The involvement of the adrenal cortex in the mammalian response to hypoxic stress was first suggested by Rabenno (23), although he concluded that the increased cholesterol content of the gland in animals exposed to high altitude was due more to the cooler ambient temperatures than to the decreased oxygen tension encountered in mountainous regions. Giragossintz and Sundstrom (13) suggested that there was a greater need by the body for adrenal cortical hormones under conditions of hypoxia. They showed that the average survival time under hypobaric atmosphere of rats treated with cortin was significantly greater than that of rats which did not receive such treatment. Interest in this problem during the Second World War (16) stimulated research which established that hypoxia is a powerful stimulator of the adrenal cortex (8, 9, 18-20, 31).

Blood lactic acid levels rise following exposure to hypoxic conditions (10) as a result of increased anaerobic metabolism. Henderson et al. (14) have shown that 8% oxygen in the inspired air is the critical level below which excess lactic acid appears in the peripheral blood. Because hypoxia causes both increased lactic acid formation and adrenal cortical discharge, studies were undertaken to determine if there is any relationship between glucocorticoid levels and lactic acid formation as related to rat survival time during hypoxic stress.

METHODS

Adult female Holtzman rats weighing 100-200 g were subjected to bilateral adrenalectomy or sham adrenalectomy by the lumbar approach and used experimentally 5-20 days later. All animals had unlimited access to Purina rat chow and normal saline. Glucocorticoid replacement consisted of a subcutaneous injection of 5.0 mg of cortisol acetate (Hydrocortone, Merck, Sharp & Dohme) 1 week prior to sacrifice and an intraperitoneal injection of 1.0 mg of cortisol sodium succinate (Soln-Cortef, Upjohn) just prior to the beginning of the experiment. Control animals received appropriate volumes of saline. Hypophysectomy by the transauricular approach (29) was performed on 180-200-g female Holtzman rats which were used 5-10 days later.

Survival time. A hypoxia chamber was constructed from a 44-liter rectangular chromatography tank and a glass plate. Holes were drilled into the plate for both inlet and outlet tubes, and airtight seals were effected at all points of contact through the use of stopcock grease. An atmosphere containing approximately 5% oxygen (equal volumes of 8 and 2% O2) or exactly 5% oxygen (premixed tank analysis) plus 0.5% CO2 in N2 was passed into the chamber at a flow rate of 1.5 liters/min. Animal survival times were determined by observing the time interval between placement of the animal in the chamber and cessation of respiratory movements. Animals were paired, e.g., an operated and a sham operated rat were both put into the chamber at the same time and not removed until both had stopped breathing. Animals with incomplete adrenalectomies or accessory adrenal tissue at autopsy were rejected. Animals with incomplete hypophysectomies were eliminated from the study following a macroscopic examination of the sella turcica.

Blood lactic acid and hematocrit. Animals were exposed to either an ambient or hypoxic (5% O2) atmosphere for 1 hr and immediately sacrificed by decapitation. The blood was collected in chilled heparinized tubes and blood lactic acid was determined on the perchloric acid deproteinated supernatant, which was incubated 30 min at 37°C with a glycine-buffered suspension of lactate dehydrogenase (LDH) to which an excess of NAD had been added. Optical density at 340 nm was read in a Beckman DU spectrophotometer against a blank of glycine-buffered LDH-NAD-perchloric.
acid (28). Blood lactic acid concentration was determined using 6.22 as the micromolar extinction coefficient for NADH. The mean microhematocrit was determined by averaging the values of three samples of free-flowing blood collected from each animal.

Statistics. Data were evaluated by the Student t test for either paired or unpaired samples. Differences in tolerance to hypoxic atmospheres in hypophysectomized and sham-hypophysectomized rats were determined by chi-square analysis.

RESULTS

Survival times. In an atmosphere containing 5% oxygen, the rats went into convulsive seizures 5–10 min prior to the cessation of respiratory movements. The seizures were followed by a short period of rigidity, after which all respiratory movements ceased. The effects of hypoxia on rats which had been previously adrenalectomized or sham adrenalectomized are shown in Table 1. Sham-adrenalectomized rats survived hypoxia an average of 101 min, while those without adrenals lived only 77 min. The difference is significant below the 0.05 level.

The effects of cortisol treatment on survival times of adrenalectomized rats exposed to an atmosphere containing 5% oxygen are depicted in Table 2. Again, in the presence of corticoids animals lived longer than paired controls which did not have replacement therapy (259 vs. 133 min, \( P < 0.025 \)). The difference in survival times of the two groups of adrenalectomized animals (saline injected or untreated) is probably due to variations in the oxygen mixture. The untreated adrenalectomized animals which survived only 77 min were exposed to a mixture of 8 and 2% \( O_2 \) run through a crude flowmeter. The resultant gas may have contained less than 5% \( O_2 \). The saline-injected adrenalectomized animals were exposed to premixed gas which was assayed at 4.96% \( O_2 \).

Sham-hypophysectomized and hypophysectomized rats showed marked differences in tolerance to an atmosphere of 5% \( O_2 \) for 1.5 hr (Table 3). Seven of the nine sham-hypophysectomized rats survived the hypoxic exposure, while none of nine hypophysectomized rats survived. Based on a chi-square distribution, the difference is significant below the 0.01 confidence level.

Blood lactic acid levels and hematocrits. Table 1 shows the effect of hypoxia on the blood lactic acid levels of sham-adrenalectomized and adrenalectomized rats. There was no difference in blood lactic acid levels of sham-adrenalectomized (1.84 
\( \mu \)moles/ml blood) and adrenalectomized (1.77 
\( \mu \)moles/ml blood) rats when exposed to ambient conditions. Exposure to hypoxia for 1 hr produced a significant rise in blood lactic acid levels of both sham-adrenalectomized (\( P < 0.001 \)) and adrenalectomized (\( P < 0.001 \)) rats. Of greater interest was the fact that the blood lactic acid level of sham-operated animals was only 8.11 
\( \mu \)moles/ml blood compared to 12.1 
\( \mu \)moles/ml blood for adrenalectomized rats, a difference significant at \( P < 0.001 \).

The effects of cortisol treatment on the blood lactic acid of adrenalectomized animals are shown in Table 5. The difference between these and the preceding values for hypoxic animals was probably due to the use of a new tank of 5% oxygen which had a slightly higher (3.48 vs. 4.73%) analyzed oxygen content. No difference in blood lactic acid levels was seen when adrenalectomized and cortisol-replaced adrenalectomized rats were exposed to ambient atmospheres (\( P > 0.10 \)). Exposure to hypoxia produced significant increases in the blood lactic acid levels in both adrenalecto-

| Atmosphere | n  | Sham Adrenalectomized, 
\( \mu \)moles/ml | \( t \) | n  | Adrenalectomized, 
\( \mu \)moles/ml | \( P \) |
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<tbody>
<tr>
<td>Ambient</td>
<td>5</td>
<td>1.84 ± 0.27*</td>
<td>&lt;0.001</td>
<td>5</td>
<td>1.77 ± 0.32*</td>
<td>&lt;0.001</td>
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<tr>
<td>Hypoxic</td>
<td>5</td>
<td>8.11 ± 1.03†</td>
<td></td>
<td>5</td>
<td>12.1 ± 1.26†</td>
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Lactic acid values are means ± se. * Sham adrenalectomized vs. adrenalectomized (\( P > 0.5 \)). † Sham adrenalectomized vs. adrenalectomized (\( P < 0.001 \)).

TABLE 5. Blood lactic acid concentrations of adrenalectomized rats exposed to ambient or hypoxic atmospheres

| Atmosphere | n  | Saline, 
\( \mu \)moles/ml | \( P \) | n  | Cortisol, 
\( \mu \)moles/ml | \( P \) |
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<tbody>
<tr>
<td>Ambient</td>
<td>5</td>
<td>9.75 ± 0.39*</td>
<td>&lt;0.001</td>
<td>5</td>
<td>1.70 ± 0.15*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypoxic</td>
<td>5</td>
<td>9.31 ± 0.46†</td>
<td></td>
<td>5</td>
<td>5.35 ± 0.29†</td>
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Lactic acid values are means ± se. * Saline vs. cortisol replaced (\( P > 0.10 \)). † Saline vs. cortisol replaced (\( P < 0.001 \)).
acid concentration of blood. Glucose can be metabolized
exposure to altitudes both produce an increase in the lactic
acids have appeared confirming that lactic acid accumulates
in both hypoxia and shock (2 1) and that tissue hypoxia has
increases during periods of anoxemia, a voluminous litera-
ture has also been shown (13) to report increased altitude tolerance with
bottoms and goetsch (5) reported an increase in the P:O
ratio of mitochondria isolated from the livers of adrenalecto-
mixed (2.73 9.31 μmoles/ml blood, P < 0.001) and cortisoltreated
adrenalectomized (1.70–5.35 μmoles/ml blood, P < 0.001) rats. Animals without glucocorticoids were again
seen to have blood lactic acid levels which exceeded the elevation found in cortisol-replaced rats (P < 0.001).
Exposure to hypoxia caused significant increases in the
hematocrit of both sham-adrenalectomized and adrenalecto-
mixed rats (Table 6). The hematocrit values were not
significantly different between sham-adrenalectomized and
adrenalectomized rats exposed to either ambient (P > 0.1)
or hypoxic (P > 0.4) atmospheres for 1 hr.

**DISCUSSION**

The role of the adrenal cortex in hypoxic stress has been
inferred from many observations that hypoxia stimulates
adrenal cortical secretion. Its protective effect in hypoxic
stress has only been shown, however, with crude prepara-
tions of adrenal cortical hormones. Giragossintz and
sunstroem (13) reported increased altitude tolerance with
dietary administration of charcoal adsorbates of adrenal
glands. charcoal alone, however, has been shown to give
protection at high altitudes (6). Adrenalectomy decreased the
altitude tolerance of animals exposed to reduced har-
mometric pressure (11). thorn et al. (30) and kotke et al. (17)
demonstrated an increase in altitude tolerance in rats and
mice injected with adrenal cortical extract. The present
study verifies and quantitates the protective effect of endoge-
nous corticosterone as well as that of specific amounts of
exogenous cortisol during exposure to low oxygen tension.
The difference between hypophysectomized and sham-
hypophysectomized rats in tolerance to hypoxia indicates that
adrenal medullary discharge alone causes no increased
survival under hypoxia. Since transauricular hypophysec-
tomy leaves the sympathetic afferents to the adrenal medulla
intact, it is assumed they are still functioning normally
during hypoxia, which is known to cause significant adrenal
medullary discharge (7). A combination of factors involving
the adrenal medulla and one or more pituitary hormones
cannot be ruled out, but seems unlikely.

Since araki (1–3) demonstrated that systemic lactic acid
increases during periods of anoxemia, a voluminous litera-
ture has appeared confirming that lactic acid accumulates
in both hypoxia and shock (21) and that tissue hypoxia has
a profound effect on the metabolic pathways of the cell.

The ischemic hypoxia of shock or the hypoxic hypoxia of
exposure to altitudes both produce an increase in the lactic
acid concentration of blood. Glucose can be metabolized
anaerobically into pyruvate which is further degraded
into water and carbon dioxide in the presence of oxygen.
Under low oxygen pressures, glucose metabolism is stopped
before pyruvic acid can be oxidized to acetyl-CoA. Entrance
to the Krebs cycle is thus blocked by the buildup of reduced
metabolites, and pyruvic acid is directed toward lactic acid
formation. The need for excess energy increases glycolysis,
augmenting the production of pyruvic acid. This forms
more lactic acid which leaves the cell, is dissolved in the
plasma fraction of the circulating blood, and is a measure of
anaerobic cellular metabolism.

The differences in blood lactic acid levels in the presence
and absence of corticoids were probably not due to shifts in
fluid compartments since the hematocrits of adrenalecto-
mixed and sham-adrenalectomized rats were statistically
indistinguishable under ambient or hypoxic conditions. The
hemocentration seen during acute hypoxic stress is probably
due to the extrusion of preformed red blood cells from the
spleen and medullary blood production centers.
The decrease in blood lactic acid secondary to hypoxia in
the presence of corticoids may reflect increased gluconeo-
genesis capacity (4), although the time periods used in these
experiments (i.e., 1 hr) is generally regarded as too short for
significant changes in gluconeogenesis to occur (22). More
likely explanations for the observed decrease in lactic acid
levels in the presence of corticosteroids are increased delivery
of available oxygen to the tissues, increased utilization of
available oxygen by the tissues, or a combination of the two.
Corticoids have been shown to increase cardiac output and
decrease peripheral resistance (24). Corticoid-induced in-
creases in cardiac ejection time and a shortening of the
isometric contraction period (15) produce an increase in
cardiac output. schumer and nyhus (26) have shown that
dexamethasone produces an increase in flow, an absence of
sludging and pooling in the microvasculature, and an in-
creased reactivity to exogenous catecholamines. These
effects would all result in an increased delivery of available oxygen to the peripheral tissues. Indirect evidence for
increased utilization of available oxygen has also been re-
ported. feigelson and feigelson (12) demonstrated increased hepatic ATP production when glucocorticoids
were administered to adrenalectomized rats. Similarly,
bottoms and goetsch (5) reported an increase in the P:O
tacitocy of mitochondria isolated from the livers of adrenalecto-
mixed rats treated with corticosterone. Increases in the
utilization of available O2 would create a larger “sink” for
reduced metabolites and as a result lactic acid production
would be decreased.

Concomitant administration of corticoids and glucose-3H
to rats in shock (26) resulted in a decrease in radioactive
labeled lactic acid and an increase in the labeling of pyruvic
acid and Krebs-cycle intermediates. These data were cor-
raborated in human (27) and primate (25) shock patients
in which infusions of corticoids were capable of producing a
decrease in systemic lactic acid.

The corticoid-induced decrease in lactic acid formation
(26) in shock as well as in exposure to hypoxia indicates that
the protective effect of corticosteroids may be exerted
through a facilitation of aerobic metabolism. Whether this
is by facilitation of oxygen delivery to the tissues or a more
efficient utilization of available oxygen is not clear. However,
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it does appear that corticoids are capable of increasing survival time of rats exposed to hypoxia while at the same time producing a significant alteration in their metabolic patterns, which results in decreased lactic acid formation.

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REFERENCES