Hindquarters vascular responses in chronically hypoxic rats

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AOKI, VINCENT S., AND SUMNER M. ROBINSON. Hindquarters vascular responses in chronically hypoxic rats. Am. J. Physiol. 217(3): 661-665. 1969.—Following 6 weeks exposure to a hypoxic environment, vascular responsiveness of the rat to lumbar sympathetic nerve stimulation (NS) and intra-arterial injections of norepinephrine (NE), epinephrine (E), and tyramine (T) was studied by autoperfusion of the hindquarters. The erythrocytosis of chronically hypoxic rats necessitated exchange transfusion with normal rat plasma to reduce blood viscosity and, consequently, perfusion pressure to levels comparable to that of controls. Under these conditions, chronically hypoxic rats showed normal responses to NS and T and increased responses to NE and E, when compared to control rats. Norepinephrine content in gastrocnemius muscles was not appreciably lowered in hypoxic rats. It is suggested that newly opened vascular channels and possibly increased availability of adrenergic receptor sites may account for the heightened response to intraarterial norepinephrine and epinephrine in chronically hypoxic rats.

adrenergic receptors; hypoxia-induced vascularity; skeletal muscle norepinephrine content; vascular responsiveness

METHODS

Groups of 20 male white rats (Charles River), weighing between 130 and 150 g, were housed for 6 weeks in a large Plexiglas decompression chamber (28 x 28 x 36 inches) maintained at 0.5 atm barometric pressure (Po2 approximately 80 mm Hg). Food and water were available ad libitum. The chamber was brought to sea level once weekly for cleaning. The rate of decompression or recompression was 30 mm Hg/min. Control rats from the same shipment were housed in pairs.

The selection of suitable controls for these experiments is complicated since the growth of chronically hypoxic rats is appreciably less than that of normoxic rats (Fig. 1) and long-term exposure to hypoxia causes a significant increase in hematocrit. Since a high hematocrit substantially increases blood viscosity (26) and results in a higher perfusion pressure at equivalent flow rates, valid comparisons of vascular responses, as measured by changes in perfusion pressure, are impossible under similar blood flow states. In order to assess the possible effects of large differences in body weight on vascular reactivity, a randomly selected group of age-matched control rats was maintained on restricted feeding (16-20 g of Purina laboratory chow daily) and their body weights adjusted so that they neither gained nor lost weight once they attained the predicted 6-week weight of the chronically hypoxic rats. The high blood viscosity of the hypoxic rats was reduced by exchange transfusion with normal rat plasma. Blood was obtained from healthy rats under ether anesthesia and the separated plasma was pooled and frozen until used. The plasma was rewarmed to 37 C prior to use. Each rat received 10 ml of plasma by exchange transfusion at a rate of 1 ml/min, using a dual syringe infusion-withdrawal pump (Harvard Apparatus). Hematocrits were measured immediately before and 15 min after transfusion. One group of control rats also underwent exchange transfusion with normal rat plasma to note the effects of the pooled plasma on vascular responses.

At the end of 6 weeks, the rats were returned to sea level and immediately prepared for autoperfusion of the hindquarters according to the technique of Brody and coworkers (4). The rats were anesthetized with pentobarbital, 50 mg/kg ip. After insertion of a tracheal cannula, neuromuscular blockade was achieved with gallamine triethiodide, 20 mg/kg iv, and controlled respiration was
then maintained by a small-animal respirator pump. Systemic blood pressure was monitored from an indwelling left carotid artery cannula. The abdominal aorta and lumbar sympathetic nerves were then exposed through a midline incision. Heparin, 5 mg/kg iv, was administered and the aorta was then cannulated below the renal arteries with polyethylene tubing (PE-90, od 0.05 inch). Blood flow was diverted via a peristaltic pump (Harvard Apparatus) into the distal aorta. The volume of the perfusion circuit was 1.3 ml. Perfusion pressure was monitored from a site near the entry of the distal cannula. Both systemic arterial and perfusion pressures were averaged electrically and continually monitored with Statham pressure transducers and a Beckman Dynograph recorder. At a constant flow, changes in perfusion pressure reflect changes in vascular resistance. Hindquarters vascular responses were elicited by supramaximal (20 v, 2 msec pulse duration) stimulation of both lumbar sympathetic nerves at the level of L2 using stainless steel bipolar electrodes and a squarewave pulse generator (American Electronic Laboratories). The duration of stimulation was 5 sec and the frequencies used were 5, 10, and 20 cycles/sec. The nerves were then severed at the L3 level and vascular responses to close intra-arterial injections of different doses of norepinephrine bitartrate, epinephrine hydrochloride, and tyramine monohydrochloride were noted. The pressor effects of tyramine are mediated through the release of endogenous norepinephrine from adrenergic neurons (5). The order of drug administration was randomized and the injectate volume was kept constant at 0.004 ml. The doses of the drugs were calculated in terms of the free base. All studies were carried out under normoxic conditions with a constant blood flow to the hindquarters of 5 ml/min.

Measurements of skeletal muscle norepinephrine content were by the trihydroxyindole method of Anton and Sayre (1). The gastrocnemius muscle was used since it was of sufficient size and easily identifiable for rapid excision. The data were analyzed by analysis of variance (27) and compared by the multiple-point bioassay method described by Finney (11). The level of significance was arbitrarily set at a P value of 0.05 or less.

Results

A comparison of the growth curve of chronically hypoxic rats with normoxic controls is depicted in Fig. 1. The starting weight of the rats was approximately 150 g. The growth of normoxic rats shows a linear increase over the 6-week period of observation. However, the growth of chronically hypoxic rats lags behind for a few weeks and then gradually increases so that, at the end of 6 weeks, their mean body weight is approximately 100 g less than that of normoxic controls. The growth curve of weight-adjusted rats is shown by the interrupted line between that of the normal-weight controls above and the hypoxic rats below. Listed in Table 1 are the base-line values of the normoxic and hypoxic groups of rats. The normoxic group includes control animals on ad libitum feeding, weight-adjusted rats maintained at the predicted weight of altitude acclimatized rats, and normoxic rats that underwent exchange transfusion. In hypoxic rats, exchange transfusion reduced the hematocrit and perfusion pressure to values comparable with those of the control and weight-adjusted groups. Mean systemic blood pressure, however, was still significantly greater in the hypoxic animals. In transfused normal rats, the hematocrit was significantly reduced. However, this was not accompanied by significant reductions in either perfusion pressure or systemic blood pressure. A summary of group differences, as compared by Tukey's test (27), is shown in the lower half of the table.

The changes in perfusion pressure following lumbar sympathetic nerve stimulation are shown in Fig. 2. The responses of the hypoxic rats were not significantly different from those of normoxic control and transfused rats, but did...
differ from responses of the weight-adjusted group. The responses of the weight-adjusted group, however, were not significantly different from those of the other two control groups.

In Fig. 3 are plotted the changes in perfusion pressure elicited by the intra-arterial injections of norepinephrine, epinephrine, and tyramine. Both norepinephrine and epinephrine produced significantly greater responses in the chronically hypoxic rats when compared to the three control groups. The responses within the normoxic groups were not significantly different. With tyramine, the responses of all groups were the same. Tyramine responses were not tested in the normoxic transfused group.

To assess the role of endogenous catecholamines in the above findings, the norepinephrine content of gastrocnemius muscles of chronically hypoxic rats was compared with those of normoxic rats (Table 2). The assumption was made that the norepinephrine content of skeletal muscle represents, in its entirety, the norepinephrine in and around the sympathetic nerve terminals (21). While the gastrocnemius muscle weights differed significantly among the three groups, total norepinephrine content in the skeletal muscle of each of the three groups was the same.

To reduce the possibility that diminished food intake and subsequent metabolic alterations in the weight-adjusted group biased the comparisons, vascular responses of a weight-adjusted group of 10 rats (mean body weight = 296 ± 6 g) were compared with those of 10 age-matched (416 ± 4 g; fed ad lib.) and 10 weight-matched (302 ± 5 g; 6 weeks younger in age than the weight-adjusted and age-matched groups) rats from the same shipment. No differences were noted in systemic blood pressure or perfusion pressure in the three groups. In addition, the vascular responses to nerve stimulation and exogenous norepinephrine, epinephrine, and tyramine were the same.

**DISCUSSION**

The mean systemic blood pressures for the three normoxic groups are lower than those encountered by Popovic and Kent (17) in anesthetized rats. However, it is not unusual to record pressures of this magnitude in the hindquarters preparation (2). The systemic blood pressure of the hypoxic group was lowered in part by exchange transfusion with plasma, possibly as a result of a reduction in blood viscosity, but nevertheless remained somewhat elevated. This observation may be explained by a difference in sensitivity to the cardiovascular depressant effects of pentobarbital after altitude acclimatization. Recent studies have demonstrated an increased rate of metabolism of another barbiturate (hexobarbital) and decreased sleeping times in mice chronically exposed to simulated high altitudes (16, 20).

Most studies reported have been concerned with vascular responses to acute hypoxia per se and not with the effects of long-term hypoxia and subsequent acclimatization on vascular responsiveness to nerve stimulation or exogenously administered agents. Carrier et al. (6) and, more recently, Detar and Bohr (10) have demonstrated that the contractility of vascular smooth muscle is directly dependent on the oxygen tension that is available to the tissue. The decrease in contractile response to epinephrine under low conditions...
oxygen tensions was shown to be completely and rapidly reversible in the isolated aortic strip preparation (10). Walker and Guyton (25) have also shown that acute changes in blood oxygen saturation influences vascular resistance in the perfused hindleg of the dog. While our experimental results might have been different had they been obtained under hypoxic conditions, it was felt that if acclimatization had indeed occurred, it should still be reflected in measurable differences in vascular responsiveness under normoxic conditions. Furthermore, previous studies performed in our laboratory, utilizing the same in vivo technique, had shown that the vascular responses to epinephrine and norepinephrine in control rats were the same during both normoxic and hypoxic situations. A summary of these data is presented in Table 3.

Our data suggest that vascular responses in chronically hypoxic rats differ from those observed in normoxic rats when both are studied under the same blood flow conditions. When compared to the weight-adjusted control group, hypoxic rats show a slightly decreased responsiveness to nerve stimulation and greatly increased responsiveness to intra-arterial injections of norepinephrine and epinephrine. This pattern of responses is also noted in cases where there is a decrease in the storage or release of norepinephrine (22). The results with tyramine, however, suggest that there was normal storage and release of norepinephrine at the nerve endings of chronically hypoxic rats. As further evidence of the availability of norepinephrine in these animals, assay of gastrocnemius muscle revealed no changes in total norepinephrine content in chronically hypoxic rats. That the observed changes in vascular responsiveness are not the result of exchange transfusion with pooled plasma is supported by the normal responses observed in normoxic-transfused animals.

When compared to the ad libitum fed controls, the differences are seen only in the increased responses to norepinephrine and epinephrine. These results are similar to those observed by Brody and Dixon (3) in alloxan-diabetic rats. In their study, it was shown that the increased responsiveness to epinephrine and norepinephrine and the normal responses to nerve stimulation and tyramine were not related to the large difference in body weight of the diabetic rats (approximately 100 g less than controls). No explanation was offered for their observations in the diabetic rat.

Thus, our data indicate that chronically hypoxic rats demonstrate an increased responsiveness to intra arterially administered norepinephrine and epinephrine, whether compared to weight-adjusted controls or to rats fed ad libitum. Two possibilities that may explain these differences are:

1. an increased sensitivity of adrenergic receptors or
2. increased availability of noninnervated adrenergic receptor sites.

In support of the first possibility are the reports that a number of agents sensitize vascular smooth muscle to the actions of intra-arterial catecholamines (9, 13, 14). In chronic hypoxia, alterations in circulating levels of electrolytes or other endogenous materials may cause such a sensitization. However, if this mechanism were operative, a preferential sensitization would have to be postulated to explain the normal responses to nerve stimulation and to injected tyramine.

A more plausible explanation for the increased sensitivity to the injected catecholamines would be the increased availability of noninnervated adrenergic receptors. Chronic hypoxia is known to cause an increase in skeletal muscle vascularity by hypertrophy and hyperplasia (8, 23). It is unlikely that a concurrent proliferation of neural elements takes place in these adult animals. It is not known whether this increase in vascularity is associated with the opening of only capillaries or also of vessels with smooth muscle components. In normoxic animals, nerve stimulation liberates norepinephrine which acts on receptor sites near the vicinity of its release; it is then quickly dissipated, primarily by reuptake by the nerve endings (18). The intravascular administration of constrictor agents would reach those receptor sites beyond the influence of the neurohormones released upon nerve stimulation (12). In the chronically hypoxic animal, the opening of vascular channels which possibly contain smooth muscle elements makes available a greater number of adrenergic receptor sites. These sites are accessible to stimulation by circulating or injected constrictor agents but are still beyond the influence of the neurohormones released upon nerve stimulation. The anatomical changes would be manifested by a normal response to nerve stimulation and injection of tyramine, but by exaggerated responses to injected norepinephrine and epinephrine. The demonstration of increased vascular responses would be expected even under normoxic conditions since it has been shown that the increase in vascularity with chronic hypoxia persists for some time after return to sea level (23).

### Table 3. Arterial blood values and hindquarters vascular responses of six control rats while breathing room air or 10% O₂ (balance: N₂)

<table>
<thead>
<tr>
<th></th>
<th>Hematocrit, vol %</th>
<th>PaO₂, mm Hg</th>
<th>PaCO₂, mm Hg</th>
<th>pH</th>
<th>Pressure, mm Hg</th>
<th>Increase in Pressure, mm Hg</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Norepinephrine</td>
<td>Epinephrine</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.03 ± 0.06</td>
<td>0.03 ± 0.06</td>
</tr>
<tr>
<td>Room air</td>
<td>42.7</td>
<td>90.3</td>
<td>38.2</td>
<td>7.38</td>
<td>92 ± 5</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>10% O₂</td>
<td>±1.2</td>
<td>±7.8 ± 1.3</td>
<td>±0.1 ± 0.02</td>
<td>92 ± 5</td>
<td>27 ± 2 ± 22 ± 2</td>
<td>39 ± 2</td>
</tr>
<tr>
<td></td>
<td>43.0</td>
<td>45.0</td>
<td>39.5</td>
<td>7.36</td>
<td>95 ± 5</td>
<td>29 ± 3 ± 37 ± 2 ± 24 ± 2</td>
</tr>
<tr>
<td></td>
<td>±1.1</td>
<td>±7.9 ± 2.0</td>
<td>±0.02</td>
<td>95 ± 5</td>
<td>29 ± 3 ± 37 ± 2</td>
<td>36 ± 2 ± 36 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± se. Mean body weight of group was 374 ± 24 g. * Values for aortic blood samples obtained using an Instrumentation Laboratory pH/gas Analyzer, model 113. † Perfusion pressure measured at a flow rate of 5 ml/min.

### REFERENCES
