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# Comparative Study of Acetazolamide and Spironolactone on Regional Blood Distribution on Exposure to Acute Hypobaric Hypoxia

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Abstract. Regional blood distribution was studied in rats, which were divided into four groups viz., (i) control, (ii) exposed to acute hypobaric hypoxia, (iii) exposed to acute hypobaric hypoxia after oral treatment with 25 mg acetazolamide, and (iv) exposed to acute hypobaric hypoxia after oral treatment with 6 mg spironolactone. The regional blood distribution was measured using radio-iodinated serum albumin ($R^{131}ISA$). The acute hypoxic exposure results in major readjustment in the blood flow to the various organs mainly from the renal and splanchic vascular beds to the heart and brain. Treatment with acetazolamide and spironolactone results only in a slight decrease in blood contents of the heart, brain and lung as compared to the hypoxia alone exposed group.

## 1. Introduction

It is well known that high altitude induces an increase in blood flow so that the oxygen delivery to tissues is maintained[1,2]. However, such a situation may not exist throughout all vascular beds. A potential mechanism for such increased blood flow would be setting of a balance, within each organ or tissue, between central autonomic produced vasoconstriction on the one hand and autonomic, metabolic and circulatory agent produced vasodilation on the other. Very limited work on regional blood distribution under acute hypoxic exposure, has been reported in the literature[3,4], however their findings are inconsistent. Certain drugs have been tried on experimental basis to ameliorate some of the symptoms of acute mountain sickness (AMS). Acetazolamide[5,6] and spironolactone[7,8] have been reported to be of some help in ameliorating the symptoms of AMS at very high altitude, but their exact mode of action is not well understood. Possibly, both these drugs affect the blood flow to the various organs and hence the oxygen delivery to them. The purpose of the present work is to investigate the regional blood distribution in various organs and tissues of rats on exposure to hypobaric hypoxia, and to see the effect of acetazolamide and spironolactone on the regional blood distribution.

## 2. Materials and Methods

Studies have been carried out in 48 male albino rats weighing 200-250 g divided into four groups of 12 each, namely (1) control, (2) exposed to acute hypobaric hypoxia, (3) oral administration of 25 mg acetazolamide with hypobaric hypoxia, and (4) oral administration of 6 mg spironolactone with hypobaric hypoxia. The hypoxic exposure was given in a hypobaric chamber maintained at 350 torr corresponding to an altitude of 6,100 m at ambient room temperature for five hours.

The animals were anesthized by an intraperitoneal injection of (35 mg/kg body wt) sodium pentabarbitol. The regional blood distribution was measured using radio-iodinated serum albumin-131 ($R^{131}ISA$). The radio-active material was diluted with 0.9 per cent sterile saline solution, and from the stock solution 0.2 ml of solution containing 1 $\mu$Ci of RISA-131 was injected through the left femoral vein. The drug was given orally to the rats in group (3) & (4), before starting the exposure. One minute after the injection, about 1.5 ml blood was drawn by cardiac puncture. Maximum amount of blood was drawn out by cutting the dorsal aorta. The lung, liver, heart, spleen, right thigh muscle, right kidney, right testes and subcutaneous tissue from the abdomen were excised, and superficial facia and fat were removed. The organ and tissues were gently blotted with filter paper and the wet weight was recorded. The brain was removed after cutting across the frontal, parietal and interparietal bones and weighed immediately. All the organs and tissues were counted in a gamma-ray spectrometer and these counts were compared wtih counts of 1.0 ml of blood hemolysed with a pint of saponin to give the blood contents of the organ.

## 3. Results

Table 1 summarised the blood contents expressed in $\mu$l per gram of wet organ weight in the various organs, namely, lung, liver, heart, subcutaneous tissue, brain, spleen, diaphragm, right thigh muscle, right testes and right kidney in all the four groups of rats : (1) control (2) exposed to acute hypoxia (3) exposed to hypoxia after treatment with acetazolamide and (4) exposed to hypoxia after treatment with spironolactone.

On hypoxic exposures splanchic blood flow, as shown by the blood contents of liver and spleen, is decreased by 25.1 per cent ($P<0.05$) and 10.4 per cent respectively. The blood contents of the lung decreased by 10.3 per cent while that of the heart increased by 32.2 per cent ($P<0.01$). Regional blood flow as shown by blood contents of kidney, decreased by 16.7 per cent ($P<0.05$). There had been a reduction in blood content by 13.8 per cent in the subcutaneous tissue ($P<0.001$), Cerebral blood flow increased by 16.4 per cent ($P<0.05$) as shown by the blood content of the brain. There was no change in the blood content of thigh muscle and testes.

Treatment with both the drugs namely acetazolamide and spironolactone, resulted in further marginal decrease in the blood content of the lungs, liver and subcutaneous tissue. The blood contents of the heart increased to a less extent in both the drug treated groups as compared to the hypoxia exposed group. Treatment with acetazolamide resulted in further elevation in blood content of the brain, as compared to hypoxia exposed group, while no change was found in spironolactone treated rats.

Table 1. Regional blood contents in rats on acute altitude exposure with or without acetazolamide and Spironolactone treatment.

| Organ | Control | Hypoxia | Hypoxia c acetazolamide | Hypoxic c Spironolactone |
|---|---|---|---|---|
| | | | (μl/g) | |
| Lung | 328.7 ±21.3 | 294.9 ±12.7 | 286.8 ±16.5 | 284.7 ±17.2 |
| Liver | 279.4 ±27.8 | 209.2* ±10.2 | 201.3* ±18.6 | 203.2* ±16.4* |
| Heart | 214.9 ±14.9 | 284.1* ±16.4 | 271.9* ±15.8 | 268.5* ±17.7 |
| Brain | 25.6 ±1.0 | 29.8* ±1.5 | 31.7* ±1.5 | 29.9* ±1.7 |
| Subcutaneous tissue | 257.0 ±2.7 | 221.6** ±3.1 | 219.6** ±3.2 | 218.9** ±3.4 |
| Spleen | 160.0 ±11.3 | 143.3 ±6.0 | 139.9 ±8.4 | 141.2 ±9.2 |
| Thigh muscle | 17.3 ±1.0 | 19.5 ±4.3 | 20.4 ±3.3 | 23.6 ±3.5 |
| Testes | 30.2 ±4.0 | 28.3 ±4.0 | 30.1 ±3.9 | 31.2 ±4.7 |
| Kidney | 263.7 ±14.3 | 219.8* ±11.5 | 259.5 ±13.1 | 268.7 ±15.3 |

*P<0.05 **P<0.001

The blood content of the kidney returned to control levels in both the drug treated rats. No significant change was found in blood contents of the spleen, thigh muscle and testes, on treatment with drugs as compared to hypoxia exposed group.

## 4. Discussion

Many workers have reported that moderate hypoxia results in an increased cardiac output[3,9–11]. Korner[11] reported that very severe hypoxia will reverse this response in the rabbit. Vogel *et al*[4] confirmed the enhancement of total blood flow together with a major readjustment of the proportion of the increased cardiac output to various tissues.

We have found a significant decrease in blood content in liver, kidney and subcutaneous tissue in the rats indicating vasoconstriction in these organs on hypoxic exposure. The lung, spleen and testes showed an insignificant decrease in their blood contents. In contrast, the heart and skeletal muscle showed significantly increased blood contents, indicating a net vasodilation in these tissues.

Our results indicated a reduction in renal blood content on hypoxic exposure and confirmed the findings as observed in anaesthetized dogs and rabbits[4,12–14]. Axelrod and Pitts[15], McDonald and Kelley[16], Caldwell *et al.*[17] and Berger *et al*[18] found renal flow to be unaffected by hypoxia. Severe hypoxia is reported to result in considerable

renal vasoconstriction and a corresponding decrease in blood flow[19]. Significant reduction in hepatic blood flow was reported by Chalmers *et al*[10] and Vogel *et al*[4], while Fischer *et al*[20] found a variable response in hepatic blood flow in the anaesthetized dogs subjected to moderate hypoxia. However, significant reduction in hepatic blood flow was reported by Chalmers *et al*[10] and Vogel *et al*[4], which is in agreement with our findings.

We have found an increased skeletal blood content in rats on acute hypoxic exposure. Vogel *et al*[4] and Black and Roddie[21] also reported large increases in blood flow to skeletal muscle during hypoxia. Kety and Schmidt[22] observed substantial increase in cerebral blood flow during brief bypoxic exposure. Kirk[23] found no change in lung blood content on hypoxic exposure using indicator dilution method, inspite of increased pulmonary arterial pressure.

The present decrease in blood content of the lungs may be attributed to a differential loss of blood on cutting of dorsal aorta because of increased pulmonary arterial pressure. *

The treatment of rats with either acetazolamide or spironolactone resulted in a marginal decrease in blood contents of the lung, liver, heart, subcutaneous tissue and spleen compared to rats exposed to hypoxia alone as both these drugs are mild diuretics. Gray *et al.*[5] reported acetazolamide to be effective in ameliorating some of the AMS symptoms due to increased $H^+$ ion concentration in the cerebrospinal fluid which influences cerebral blood flow and results in a greater degree of hyperventilation, resulting in higher $pO_2$ and lower $pCO_2$. Laux and Raichle[6] found that acetazolamide produces an immediate and significant increase in cerebral blood flow, which partially compensated reduced tissue oxygen availability. We have also observed a slight increase in blood contents of the brain. However, no change occurs in blood content of brain on treatment with spironolactone. Both these drugs resulted in restoring the normal blood contents of the kidney due to their diuretic action. The decreased blood content of the lungs may be helpful in ameliorating pulmonary edema.

In conclusion, the cardiovascular response to acute hypoxic exposure results in the major readjustment of the blood flow to the various tissues. The blood from renal and splanchic vascular beds was diverted to the heart and brain.

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