Original Research

Effects of Cold Air on Serum Catecholamine Levels in Patients with Cardiovascular or Cerebrovascular Disease

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Abstract

This study analyzes the effects of cold air on catecholamine (CA) levels. The CA levels of patients with cardio-cerebrovascular diseases, healthy people, Wistar rats, and spontaneously hypertensive (SHR) rats were determined before, during, and after cold air activity. The levels of dopamine (DA), norepinephrine (NE), and adrenaline (AD in humans and experimental animals changed in all three periods of cold air activity. The change in DA levels was statistically significant ($P<0.05$). The DA, NE, and AD levels in the controls and the Wistar rats increased during cold air activity and decreased after cold air activity. The variation in CA levels was not exactly the same between the SHR rats and the cardio-cerebrovascular disease patients. The special meteorological conditions caused by cold air affects CA secretion, which induces the occurrence, development, and outcome of cardio-cerebrovascular diseases. Moreover, the results of the animal experiments could not be directly extrapolated to humans.

Keywords: cold air, catecholamine, cardio-cerebrovascular diseases, wistar rat, SHR rat

Introduction

Cardiovascular and cerebrovascular diseases are common diseases that influence human health. Previous studies indicated a significant correlation between severe temperature changes and the onset of cardio-cerebrovascular diseases. The effects of cold air have been studied among patients with cardiovascular and cerebrovascular disease patient [1-4]. Epidemiologic and animal experimental studies indicate that blood pressure of humans and animals increases upon exposure to cold air. Thus, cold air is considered one of the major factors in cardio-cerebrovascular diseases [5-7]. However, blood pressure changes are regulated by many hormones; catecholamines (CA), for example, significantly increase or decrease blood pressure [8, 9]. Thus, CA plays an important role in the occurrence, development, and outcome of cardio-cerebrovascular diseases. The effects of temperature change on CA level and, subsequently, cardio-cerebrovascular diseases have not been studied. In this study, we simulated the effect of cold air on the CA levels in humans and experimental animals to analyze the occurrence, development, and outcomes of cardio-cerebrovascular diseases. Several primary studies have been conducted in animals, but not on humans. Animal experimental results could not be directly extrapolated to humans. The current study applied the same conditions on humans and experimental animals, and the same indices were measured. The results were analyzed and compared.

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Zhangye City, Gansu Province was chosen as the crowd field study site because cold air regularly passes through this city [10, 11]. The CA levels of patients with cardio-cerebrovascular diseases and that of healthy subjects were observed during moderate strength cold air in 2011. This typical cold air activity was simulated in an artificial climate box wherein Wistar rats and spontaneously hypertensive (SHR) rats were fed. The changes in CA were then measured. The mechanism of cardio-cerebrovascular disease induction by cold air was discussed. Furthermore, the feasibility and reliability of extrapolating the animal experimental results to the human situation were analyzed. This study provides data and theoretical evidence of the prevention of cardio-cerebrovascular diseases and medical meteorology.

Materials and Methods

Population Epidemiologic Study

The subjects were chosen from Zhangye City, an area with prevailing cold winds west and northwest of China that experiences major temperature changes. Zhangye City has no industrial pollution, and the air quality conforms to the secondary standard of environmental air quality. The random Chester sampling method was used to choose the study subjects. The subjects were 40 to 70 years old, living within 1,000 m of the monitoring points. The patients had cardio-cerebrovascular diseases with no history of alcoholism, smoking, and organic diseases, and had not taken any drugs for cardiovascular and cerebrovascular diseases within the previous 3 days. The patients were subjected to health screening and haematological examinations. A total of 36 cases and 50 healthy people for the control group were chosen according to the same selection standards for patients with cardio-cerebrovascular diseases. Diet, sleep, and activities were recorded daily. The subjects who showed poor compliance and non-conformance were excluded from the experiment. The final data included 30 patients with cardio-cerebrovascular disease (16 male, 14 female) and 40 controls (24 male, 16 female). The cardio-cerebrovascular diseases included in this study were cerebral thrombosis, cerebral haemorrhage, hypertension, coronary disease and myocardial infarction. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Lanzhou University. Written informed consent was obtained from all participants.

Meteorological Data of Field Experiment Collection

The cold air activity data (March 10 to 15), which included air temperature and air pressure, were provided by the Lanzhou Central Meteorological Observatory. The cold air was graded based on the grading of cold air in China (GB/T20484-2006) [5].

Catecholamine Measurement in Humans

Fasting venous blood (5 ml) was drawn from subject's arms 24 h before cold air activity, during cold air activity (minimum temperature), and 24 h after cold air activity. The blood was centrifuged and stored at -80ºC. The serum was analyzed using enzyme-linked immunosorbent assay kits (ELISA kits; R&D Systems China Co., Ltd., Shanghai, China) after the experiment.

Experimental Animal

Up to 30 Wistar rats and 30 SHR rats (Vital River Laboratory Animal Technology Co. Ltd.; SCXX, Peking, China) were used in this study. The male-to-female ratio was 1:1. The animals were 8 weeks to 10 weeks old. The difference in mean weight between the Wistar rats and the SHR rats was not statistically significant ($P>0.05$). This study was performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Lanzhou University.

Meteorological Data for Animal Experiments

Experiment temperatures were increased to 12.1ºC compared with the real temperatures of cold air activity. The minimum temperature was 5ºC, and the experimental temperatures were carried out in an artificial climate box (GDJS-500L, Pulingte Co., Tianjin, China).

Simulation Method of Cold Air Activity

Cold air activity was simulated using an artificial climate box. The experimental animals were fed for 20 days in an artificial climate box with 60% relative humidity at 21ºC. The SHR and Wistar rats (female: male ratio of 1:1) were randomly divided into groups A, B, and C.

Catecholamine Measurement in Experimental Animals

Each group of experimental animals was taken out separately during each period of cold air activity. The experimental animals were anesthetized using chloral hydrate. Afterward, 2 ml of blood was drawn from the animals through the abdominal aorta. The measurement method was the same as for people.

Statistical Analysis

The mean values between the patients and the control group were analyzed using the Mann-Whitney test. The data during cold air activity were examined using Kendall's W test. Statistical analysis was performed using SPSS 11.0 software (SPSS Inc., Chicago, USA). Differences with P values < 0.05 were considered significant.

Table 1. Meteorological data of cold air activity of Zhangye (ºC).

Variable	11th	12th	13th	14th	15th
$Tmax_{24}$	15.1	14.0	4.8	-1.3	3.5
$Tmin_{24}$	-4.1	-1.0	-6.1	-7.4	-12.0
$\Delta Tmin_{48}$	6.4				

$Tmax_{24}$ is the maximum temperature of one day (24h), $Tmin_{24}$ is the minimum temperature, $\Delta Tmin_{24}$ is the difference between the highest $Tmin_{24}$ and the lowest $Tmin_{24}$.

Results

Cold Air Activity Characteristics

The minimum temperature in Zhangye City was -1.0ºC on 12 March 2011 and -1.0ºC on 14 March 2011. The minimum temperature dropped 6.4ºC. According to the grading of cold air in China (GB/T20484-2006), moderate strength cold air denotes a minimum temperature drop of 6ºC to 8ºC for 48 h (Table 1, Fig. 1).

Basic Characteristics of Study Objects

The following study group characteristics were gathered according to the access standard: the patient group had 30 cases, with a sex ratio of 1:1. The average age of the subjects was 59 years old. Up to 6 cases had cerebral thrombosis, 2 cases had cerebral haemorrhage, 12 cases had coronary heart disease, and 10 cases had hypertension. The control group consisted of 40 healthy subjects, with an average age of 55 years and a sex ratio of 3:2. The differences in sex and age structure between the patient group and the control group were not statistically significant ($P > 0.05$) (Table 2).

Catecholamine Levels of Population

Compared with the control group, the dopamine (DA), norepinephrine (NE), and adrenaline (AD) levels in the patient group had no significant changes ($P>0.05$) during the same period of cold air activity. The DA levels in the patient group and the control group during the second and third periods of cold air activity significantly changed ($P<0.05$) compared with the levels before cold air activity. However, the AD and NE levels had no significant changes ($P>0.05$).

The DA, AD, and NE levels in the control group during cold air activity were higher than those before cold air activity, and the changes in DA levels were statistically significant ($P<0.05$). The AD and NE levels in the patient group increased during cold air activity, whereas the DA level decreased during cold air activity. The changes in the patient group were not statistically significant ($P>0.05$).

The DA levels in the control group decreased after cold air activity and were lower than the levels before and during cold air activity. The AD and NE levels decreased, but were higher than those before cold air activity. The changes in these three indicators were all statistically significant ($P<0.05$) compared with the levels before cold air activity. The DA levels in the patient group were significantly decreased compared to those in the other two periods. Compared with the level before cold air activity, the AD and NE levels significantly increased during and after the cold air activity ($P<0.05$).

The DA levels in the control group significantly changed during the cold air activity ($P<0.05$) compared with those before the cold air activity. The DA and NE levels of the patient group significantly changed ($P<0.05$; Table 3).

Variation Trend of Temperature in Simulated Experiment

To ensure that the minimum temperature in the experiment was 5ºC, the experimental temperatures were all increased to about 12.1ºC compared with the actual temperatures. The meteorological data was input into the artificial climate box, which simulated the variation trends in temperature (Fig. 2).

CA Levels in the Wistar Rat Group and the SHR Rat Group

The NE levels in the SHR and Wistar rat groups before cold air activity were different. The changes in the three CAs in the SHR rat group during cold air activity and after cold air activity were not statistically significant compared with the Wistar rat group. The changes in the DA level of the Wistar rat group during the three stages of cold air activity was statistically significant ($P<0.05$). The DA level during

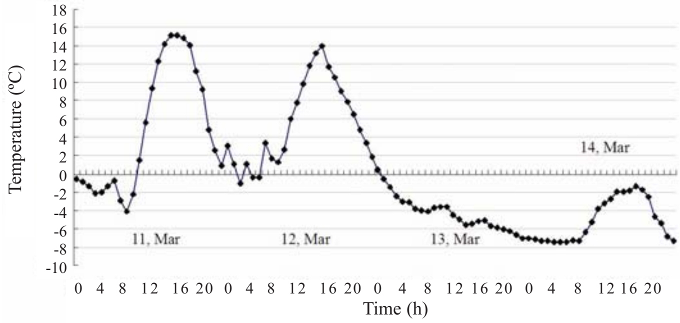

Fig. 1. Temperature changes from March 11-14, 2011 in Zhangye City.

Table 2. Objects' basic sex and age characteristics.

Group	N	Sex (%)		Age (%)			
		Male	Female	40-	50-	60-70	$\bar{x}$±s
Control	40	24 (60.0)	16 (40.0)	14 (35.0)	11 (27.5)	15 (37.5)	55±9.8
Patient	30	16 (53.5)	14 (46.7)	9 (30.0)	9 (30.0)	12 (40.0)	59±10.0
Total	70	40 (57.1)	30 (42.9)	23 (32.9)	20 (28.6)	27 (38.6)	57±9.6

Table 3. Catecholamine levels of population (median, ng/L).

Period	Control group			Patient group		
	DA	NE	AD	DA	NE	AD
Before cold air activity	344.1	173.5	68.8	715.41	159.93	76.01
During cold air activity	425.60[a]	275.41	80.25	693.68	305.97	79.25
After cold air activity	64.53[ab]	268.61[a]	78.25[a]	67.31[ab]	373.28[ab]	80.5
Significance test	χ^2=13.317	χ^2=0.454	χ^2=3.659	χ^2=8.222	χ^2=4.056	χ^2=1.185
(Mann-Whitney U)	P=0.001	P=0.797	P=0.161	P=0.016	P=0.132	P=0.553

[a] P<0.05 compared with before cold air activity, [b] P<0.05 compared with during cold air activity.

cold air activity was significantly increased compared with that before cold air activity (P<0.05). The NE and AD levels increased, but these changes were not statistically significant. The DA levels in the Wistar rat group significantly decreased after cold air activity, and the NE and AD levels significantly increased (P<0.05) compared with those before cold air activity.

The DA and NE levels in the SHR rat group significantly decreased (P<0.05) during cold air activity compared with the levels before cold air activity. The AD level in the SHR rat group increased after cold air activity, whereas the NE and AD levels continuously decreased (Table 4).

Discussion

CA, which is secreted by the adrenal medulla, is a neurotransmitter that contains catechol and amine. CA is derived from the L-amino acid tyrosine in sympathetic nerves, the adrenal medulla, and chromaphil cells. CAs include NE, AD, and DA. The adrenal medulla is not an essential organ, but plays an important role in systemic stress response [12, 13]. Intense physiologic or pathologic stimuli could initiate CA release into the blood to act on the biological system and cause a series of physiological effects, such as blood vessel and myocardial contraction or diastole. Thus, CAs are closely connected with cardiovascular and cerebrovascular diseases. A small amount of CA is released into the blood during normal body conditions; however, the CA levels increase when the external environment (temperature, air pressure, psychology, and so on) affect the body [14-16]. Previous studies have suggested that varying meteorology is one of the major factors that affect cardiovascular and cerebrovascular diseases. Severe climate changes are closely related to the onset of cardiovascular and cerebrovascular diseases, and cold air activity increases the morbidity and mortality of cardiovascular and cerebrovascular diseases [17-19]. Cardiovascular and

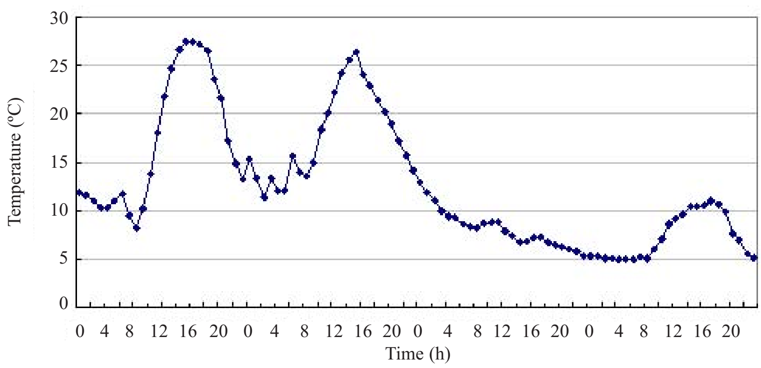

Fig. 2. Simulating the variation trend of temperature.

Table 4. Catecholamine levels of Wistar rat group and SHR rat group (median, ng/L).

Period	Wistar group			SHR group		
	DA	NE	AD	DA	NE	AD
Before cold air activity	75.49	49.39	60.90	81.68	112.84	68.74
During cold air activity	92.99[a]	86.1[a]	78.72[a]	72.1[a]	55.59[a]	66.05
After cold air activity	79.01[b]	74.62	71.54	76.03	50.3	61.46
Significance test	χ^2=5.585	χ^2=2.363	χ^2=2.216	χ^2=5.478	χ^2=5.291	χ^2=0.407
(Mann-Whitney U)	P=0.038	P=0.307	P=0.330	P=0.065	P=0.071	P=0.816

[a] P<0.05 compared with before cold air activity, [b] P<0.05 compared with during cold air activity.

cerebrovascular diseases are negatively correlated with changes in meteorological conditions, such as air temperature and air pressure [19, 20]. Studies on the effects of temperature change on CA release in healthy people and patients with cardio-cerebrovascular diseases are significant in revealing the effect of weather variations on the occurrence, development and outcome of cardio-cerebrovascular diseases. This study conducted animal experiments and monitored population CA biochemical indices to investigate the changes in the level of NE, AD, and DA in humans and animals during cold air activity. This study revealed the effect of cold air exposure on the status of cardio-cerebrovascular diseases at the biochemical and metabolic levels. Moreover, the differences between population results and animal experimental results were compared to determine a physiological basis for the effect of cold air on cardio-cerebrovascular diseases and to provide evidence for the prevention and treatment of cardio-cerebrovascular diseases.

In actual cold air activities, moderate strength cold air denotes that the minimum temperature dropped by 5.1ºC for 24 h or by 6.4ºC for 48 h. Cold air activity often occurs in northwestern China. This study indicated that the DA levels in people and experimental animals changed in cold air activity. The DA levels in the healthy controls and the Wistar rat group increased during cold air activity, but returned to normal levels after the cold air activity. The DA level in the patient group continuously decreased during cold air activity and after cold air activity, but the NE and AD levels continuously increased. The DA level in the SHR rat group decreased during cold air activity and then increased after the cold air activity. The NE and AD levels in the SHR rat group continuously decreased. Our results indicated that organisms secrete more adrenaline, which accelerates blood circulation to combat the cold when people and animals are exposed to cold conditions. However, increased adrenaline induces vasoconstriction, which increases blood pressure and the risk for cardio-cerebrovascular disease. This finding is consistent with epidemiological studies that found that cold air results in increased morbidity and mortality among patients with cardio-cerebrovascular diseases. Our study provides sufficient evidence to prove that cold air increases morbidity and mortality among patients with cardio-cerebrovascular diseases. This study also indicated that the experimental results are not consistent between animals and humans, thereby suggesting that animal experimental results cannot be randomly extrapolated to humans.

On one hand, this study confirms that cold air activity increases catecholamine release, which influences cardiovascular and cerebrovascular contraction and relaxation and results in the occurrence, development, and outcome of cardio-cerebrovascular diseases. On the other hand, cold air does not directly cause cardio-cerebrovascular diseases, but it can cause neurotransmitter (as CA) dysfunctions. Cold air decreases the self-regulatory adaptability to the changing environment; thus, CA synthesis and metabolism does not return to normal under cold air stimulation. Therefore, the NE and AD levels in the control group could return to levels before cold air activity, whereas those in patients with cardio-cerebrovascular diseases remained high or continued to increase. This difference is due to the stronger self-regulatory capabilities of the controls compared with the patients, which exacerbates the effects of cold air on the cardio-cerebrovascular system. This mechanism could explain the increased mortality of cardio-cerebrovascular diseases after cold air activity.

Wistar and SHR rats are favourable animal models for hypertension and atherosclerosis. However, results were not completely the same between animal experiments and population investigation. Nevertheless, the total change in CA was similar. Cold air clearly affected the DA levels in humans and experimental animals, which indicated that DA is a sensitive indicator of the effects of cold air on cardio-cerebrovascular diseases. Moreover, the changes in the levels of DA, CA, and NE were the same in the control and Wistar rat groups before, during, and after cold air activity. Thus, the levels of DA, CA, and NE increase during cold air activity and decreased after cold air activity. However, the change in CA between patients with cardio-cerebrovascular disease and the SHR rats were significantly different. This difference could be accounted for as follows:

1) The response of the body to cold air is different between humans and animals and the study groups were not exposed to exactly the same conditions,
2) The moderate temperature simulation acted on experiment animal groups,
3) The SHR rat group included only hypertensive rats,

4) The number of humans and experimental animals included in the study was limited,
5) The humans and experiment animals in this study are different.

Therefore, the results of the animal experiments could not be extrapolated to humans. Similar studies that control the interfering factors with appropriately increased sample sizes should be conducted.

The result of our study is significant and complements previous results and standpoint. This study could also explain the epidemiologic survey results on cold exposure and provide evidence for further studies on the pathogenesis of cardio-cerebrovascular diseases. Improving the CA levels could prevent cardio-cerebrovascular diseases induced by rapid temperature changes, as well as reduce the medical burden and improve health.

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