The Effect of Trigeminal Neurotomy on the Alteration of Local Cerebral Blood Flow of Normotensive and Hypertensive Rats in Acute Cold Stress

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Abstract

The cold season is reported to have the highest incidence of stroke in a year. Cold is usually detected by cold receptors in the face. The present studies were designed to test whether the trigeminal nerve plays a role in the regulation of local cerebral blood flow (LCBF) in animals exposed to a cold environment. Since hypertension affects the incidence of strokes, both Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were used. Each species was divided into four groups: trigeminal neurotomy (TNx) or control group at room temperature (20°C) or cold environment (5°C), respectively. LCBF in 14 brain structures was measured using the [^{14}C] iodoantipyrine technique and tissue dissecting methods. Our results show that TNx did not alter physiological parameters and LCBFs in WKY and SHR kept either at 20°C or at 5°C for 30 min. However, a transient exposure (30 minutes) to cold caused concomitantly a significant decrease in core body temperature of both WKY and SHR groups ($p<0.05$, Student's paired t-test) and a significant decrease in LCBFs at the temporal cortex (TC), hypothalamus (HYP) and midbrain (MID) of WKY and TC of SHR ($P<0.05$, MANOVA). TNx did not alter LCBFs significantly following transient cold exposure in WKY and SHR. Our findings indicate that in the cold environment, the lowered LCBFs in some areas of the brain may relate to the decreased metabolic rate caused by decreased body temperature, and may partly contribute to the higher prevalence of stroke in winter. Our findings also suggest that trigeminal nerve do not exert tonic control of LCBFs and the cold afferents in trigeminal nerve are not important in modulation of the LCBFs.

Key Words: cold stress, local cerebral blood flow, normotensive rat, spontaneous hypertensive rat, trigeminal neurotomy

Introduction

The prevalence of stroke has been reported to be high in winter as compared with that in other seasons (2-5, 8, 9, 12, 16, 18, 20, 23, 25, 28). The pathophysiology of this seasonal peak of strokes is still not well known. In winter, the face is the most commonly exposed site of the human body to cold air since the body is covered with clothing. Local stimulation of the face and nasal-oral respiration flow

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by cold air or wind may elicit bradycardia as well as an elevation of blood pressure (1, 17, 30). Therefore, the trigeminal nerve may play a role in the cardiovascular response in cold weather. Recently, some evidences show that cerebral vessels are densely innervated by a calcitonine gene related peptide (CGRP) and substance P containing nerves arising from the trigeminal ganglion (7, 11, 15, 19, 27). These two peptidergic nerves can induce a remarkable cerebral vasodilation by axon reflex-like mechanism when the trigeminal sensory nerves are stimulated. Thus, it is hypothesized that the local cerebral blood flow (LCBF) of the adult normotensive and spontaneously hypertensive rats will be altered when these animals are temporarily exposed to cold air (14, 21, 33). In this study, the entire animal was exposed to a cold environment instead of local facial stimulation because rats are heavily covered with heavy fur while their faces are sparse. This experimental design is similar to humans exposing themselves in winter, namely, in that the human has clothes on the body with the face in direct contacting with cold air.

Materials and Methods

Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) about 52 weeks old were used. This age of rats is pretty much compatible with that of adult humans who might easily suffer from stroke. Animals of each species were divided into 4 groups. 1) rats having a previous sham trigeminal neurotomy (TNx) kept at room temperature (20°C). 2) rats having a previous sham TNx exposed in a cold room (5°C) for 30 minutes. 3) rats previously treated with bilateral TNx kept at room temperature. 4) rats previously treated with bilateral TNx and exposed to a cold (5°C) environment for 30 minutes. At the end of an exposure to various temperatures, the LCBF in various brain areas was measured by ^{14}C-iodoantipyrine and tissue dissection.

Trigeminal Neurotomy (TNx)

At the beginning of surgery, the rats were anesthetized with ketamine (120 mg/kg, intraperitoneal injection). The skin over the head was prepared with beta-iodine. A scalp incision about 5 mm was made near the orbital rim. Intraorbital structures were gently retracted laterally, and the structures in the ethmoidal foramen were exposed under the microscopic field (26). The nasociliary nerve was well identified under high magnification and then carefully transected just proximal to the ethmoidal foramen. Meanwhile, the fine membranous structure containing the post-ganglionic fibers from the sphenopalatine ganglion located just below the nasociliary nerve was well perserved. Rats of each species were randomly divided into the neurotomy or sham operation group. For the neurotomy rats, the ophthalmic branch of the trigeminal nerve was cut just outside the ethmoidal foramen. For the sham operation group, this nerve was exposed but not cut. Then, the skin was sutured in layers. An identical procedure was performed on the opposite side. After the operation, the rats were returned to the cage. Two weeks later, the rats were subjected to various temperature exposures and their LCBF was measured.

Cold Stress and Local Cerebral Blood Flow Measurement

Before the experiments, the rats were starved for about 15 hours. The animals were anesthetized using a gas mixture of halothane and oxygen. Cannulas were placed in the femoral arteries, bilaterally, for the recording of arterial pressure as well as the withdrawing of blood, and in a femoral vein for injection of the radiotracers. The surgical wounds were infiltrated with lidocaine hydrochloride jelly and closed with sutures. A plaster cast was fitted below the midthorax of each rat to immobilize the hindlimbs and to protect the catheters. Anesthesia was discontinued, and body temperature of each rat was kept around 37°C by a heat lamp. They were allowed at least 2 hours to recover before beginning the cold stress and the LCBF measurement. This 2 hours recovery time was sufficient to allow the rats to regain their normal physiological state (13, 32). The neurotomy and sham-operation animals were then divided randomly into the cold or room temperature group. Animals in the cold temperature group were placed in a cold room for 30 minutes. Their arterial blood pressure, heart rate, and body temperature were continuously monitored and recorded (RS 3200, Gould Instrument Systems, Inc., OH, USA) throughout the entire experimental procedure. The other physiological variables were determined by measuring arterial blood gases and plasma glucose at the end of the exposure period.

These rats were then subjected to LCBF measurements. For each rat, approximately 12 μCi of IAP in 1 ml of normal saline was infused at a constant rate via the femoral venous catheter for a period of 30 seconds, during which arterial blood samples were collected every 5 seconds for assay of arterial concentration of IAP (13, 31). The animals were decapitated at 30 seconds, and their brains were quickly removed and then immersed in isopentane chilled to -40°C. The brains were placed on a dissecting plate kept in a freezer at -10°C.

Blood samples were centrifuged and plasma ^{14}C-radioactivity was determined by beta counting

Table 1. Physiological Parameters of Sham Operated and Trigeminal Neurotomy (TNx) in Wistar-Kyoto Rats (WKY) and Spontaneously Hypertensive Rats (SHR).

Groups	WKY				SHR			
	Sham		TNx		Sham		TNx	
Parameter	Pre-cold	Post-cold	Pre-cold	Post-cold	Pre-cold	Post-cold	Pre-cold	Post-cold
Body temperature (°C)	37.0±0.6	35.1±0.7[a]	37.2±0.4	34.9±0.4[a]	37.4±0.1	36.1±0.1[a]	37.2±0.2	35.4±0.2[a]
Body weight (g)	418±15		424±26		373±7		371±5	
MABP (mm Hg)	126±1	125±7	125±3	117±5	164±4	168±4	165±4	168±4
HR (beats/min)	374±21	354±19	412±13	360±16[a]	358±10	408±5[a]	354±10	394±6[a]
Blood glucose (mg/dL)	189±21	182±26	203±25	212±20	150±13	198±13[a]	153±13	180±10[a]
pH	7.46±0.01	7.44±0.01	7.49±0.01	7.43±0.01	7.47±0.01	7.46±0.01	7.46±0.01	7.44±0.01
$PaCO_2$ (mm Hg)	36.9±0.8	35.6±1.1	36.5±1.5	39.0±1.3	36.9±0.6	36.2±1.8	37.3±1.6	37.6±1.5
PaO_2 (mm Hg)	81.3±3.9	96.2±6.6[a]	75.4±8.0	84.9±9.8[a]	85.9±2.9	96.7±4.2[a]	87.8±2.1	103.5±3.7[a]
O_2 sat (%)	96.3±0.7	96.9±0.9	95.1±1.2	95.4±1.4	97.0±0.3	97.6±0.4	97.1±0.3	98.1±0.3[a]
Hct (%)	41.9±1.3	42.3±1.6	45.0±2.6	45.3±2.4	44.2±1.1	44.5±0.9	42.6±1.6	44.1±0.9
Hgb (g/dL)	13.9±0.5	14.0±0.5	15.1±0.9	15.1±0.8	14.8±0.4	14.8±0.3	14.2±0.5	14.8±0.3

MABP: mean arterial blood pressure, HR: heart rate, $PaCO_2$: partial pressure of CO_2, PaO_2: partial pressure of O_2, O_2 sat: saturation of oxygen, Hct: hematocrit, Hgb: hemoglobin. Data are presented as mean±SEM, [a] $p<0.05$ when compared with pre-cold state (Student's paired t-test).

(Tri-Carb 1500, Packard Instrument Company, IL, USA). By referring to a standard anatomical atlas, brain tissue samples of bilateral hemispheres were dissected from the following 14 areas: the frontal cortex (FC), sensorimotor cortex (SMC), temporal cortex (TC), occipital cortex (OC), caudate putamen (CP), hippocampus (HIP), thalamus (THA), hypothalamus (HYP), superior colliculus (SC), inferior colliculus (IC), ventral midbrain (MID), pons, medulla oblongata (MED), and vermis (VER). The 14 brain sections were weighed, dissolved in 0.5 ml of 1.0N sodium hydroxide, vortexed, hydrolyzed for 15 hours at 40°C in a shaking water bath, and neutralized with 1.0N hydrochloric acid before adding scintillant. The ^{14}C-radioactivity of each brain tissue sample was determined by beta counting. The LCBF for each brain structure was calculated according to the Kety-Sokoloff equation, brain and plasma radioactivity (13, 31).

Statistical Analysis

All physiological data were presented as mean±SEM. Differences in physiological data before and after the rats were put into the cold room were assessed with the Student's paired t-test. Data differences between the sham and neurotomy groups as well as between WKY and SHR groups were analyzed by analysis of variance (ANOVA).

Data of LCBF in 14 structures were presented as mean±SEM. Differences between the groups were assessed by the multivariate analysis of variance (MANOVA). When the MANOVA assessments between groups differed significantly, the univariate F-tests were used to highlight the brain structures where the differences were significant. A significant difference in the statistical results was designated as $p<0.05$.

Results

Table 1 shows the physiological parameters of the sham and TNx groups in WKY and SHR rats. The physiological parameters include mean arterial blood pressure (MABP), heart rate (HR), blood glucose, arterial pH, partial pressure of CO_2 ($PaCO_2$), partial pressure of O_2 (PaO_2), oxygen saturation (O_2 sat), hematocrit (Hct), hemoglobin (Hgb), core body temperature, and body weight (Wt).

The body weights of SHRs were all significantly less ($p<0.05$) than those of WKYs. This is compatible with the previous report (32). After a transient exposure to the cold room (5°C), both WKY and SHR in the sham and TNx groups showed a significant ($p<0.05$) decrease in core body temperature as compared to their pre-cold state. However, the magnitude of decrease in core body temperature after cold exposure was similar in all 4 groups and ranged from 1.3-2.3°C.

The mean blood pressure was similar in the sham and TNx groups in both WKY and SHR. After a 30-minute exposure to the cold environment, both

Table 2. Local Cerebral Blood Flow of Sham Operated and Trigeminal Neurotomy (TNx) in Wistar-Kyoto Rats (WKY) and Spontaneously Hypertensive Rats (SHR).

Groups	WKY				SHR			
	Room		Cold		Room		Cold	
Parameter	Sham	TNx	Sham	TNx	Sham	TNx	Sham	TNx
FC	197±15	171±13	195±15	186±16	190±20	153±5	198±18	172±10
SMC	198±17	161±8	204±19	193±16	189±21	167±12	220±19	187±10
TC	88±5	92±9	72±6	65±6	117±12	104±6	91±7	86±6
OC	159±11	142±10	146±8	152±14	157±16	131±4	164±12	146±9
CP	77±4	74±3	76±4	71±4	77±9	66±3	77±5	70±6
HIP	87±5	82±7	85±6	84±8	80±6	63±2	81±4	80±6
THA	109±6	106±6	105±7	102±8	104±9	88±3	90±6	83±3
HYP	75±5	85±7	76±9	56±6	61±6	63±4	75±7	71±7
SC	123±8	116±9	111±8	108±8	102±8	87±3	106±5	94±8
IC	112±7	97±5	106±6	91±8	97±6	79±2	88±6	82±5
MID	88±4	84±4	78±4	72±5	76±5	68±2	72±4	65±3
PONS	77±4	74±4	78±3	66±4	72±6	59±2	69±4	60±2
MED	70±3	65±7	74±4	65±4	54±5	50±3	56±3	48±2
VER	90±6	91±2	87±5	81±6	94±7	76±3	83±5	75±2

FC: frontal cortex, SMC: sensorimotor cortex, TC: temporal cortex, OC: occipital cortex, CP: caudate putamen, HIP: hippocampus, THA: thalamus, HYP: hypothalamus, SC: superior colliculus, IC: inferior colliculus, MID: ventral midbrain, MED: medulla oblongata, VER: vermis. Data (in milliliter per 100 grams per minute) are presented as mean±SEM of eight rats in each group.

WKY and SHR did not display any change in blood pressure. The heart rate of the sham group of WKY showed no change after cold stress. In the TNx group of WKY, cold exposure resulted in a significant decrease ($p<0.05$) in heart rate (412±13 vs. 360±16, $p<0.05$). In contrast, for both the sham and TNx groups of SHR, cold stress led to significant increases in heart rate (358±10 vs. 408±5 for the sham group, 354±10 vs. 394±6 for the TNx group). The plasma glucose levels were also significantly elevated after cold stress ($p<0.05$) in both the sham and TNx groups of SHR, but not for the two groups of WKY. The arterial pH and CO_2 content displayed no significant change after cold stress for both groups of WKY and SHR. However, arterial oxygen content became higher ($p<0.05$) in a cold environment for both groups of WKY and SHR. This might be due to a relative higher oxygen content in a cold environment as compared to that of a room-temperature environment. The oxygen saturation in arterial blood showed no change for both WKY groups and the sham group of SHR. In the TNx group of SHR, however, arterial oxygen saturation was mildly elevated ($p<0.05$) in a cold environment. The systemic Hct and Hgb levels did not alter after a transient cold stress.

Table 2 shows the data of LCBFs of the 8 groups of WKY and SHR. In WKY, LCBFs in 14 brain areas of the sham-room group ranged from 70±3 ml/100g/min in the medulla oblongata to 198±17 ml/100g/min in the sensorimotor cortex. These values were similar ($p>0.05$) to those of the TNx-room group of WKY by MANOVA analysis. In SHR, LCBFs in brain structures of the sham-room group ranged from 54±5 ml/100g/min in the medulla oblongata to 190±20 ml/100g/min in the frontal cortex. These values were also similar ($p>0.05$, MANOVA) to those of the TNx-room group of SHR. Data of LCBFs between the sham-room and sham-cold groups were similar ($p>0.05$, MANOVA), for either WKY or SHR. LCBFs in 14 brain areas were also similar ($p>0.05$, MANOVA) in the TNx-room and the TNx-cold groups for either WKY or SHR. Since LCBFs were similar between sham-room and TNx-room of WKY, the two sets of LCBFs were lumped together. Similarly, the LCBFs of sham-cold and TNx-cold groups were put together. Data were then compared between the two sets of lumped data to measure the effects of temperature on the LCBF changes in WKY (Fig. 1). The results show that LCBFs at the TC, HYP, and MID areas were significantly ($p<0.05$, MANOVA) lower in the cold environment as compared to those of the room-temperature environment. The percentages of decrease were 24% (TC), 18% (HYP) and 13% (MID). The above-mentioned comparison was performed for SHR (Fig. 2). LCBF of TC was 20% less ($P<0.05$, MANOVA) in the cold environment than in the room-temperature environment.

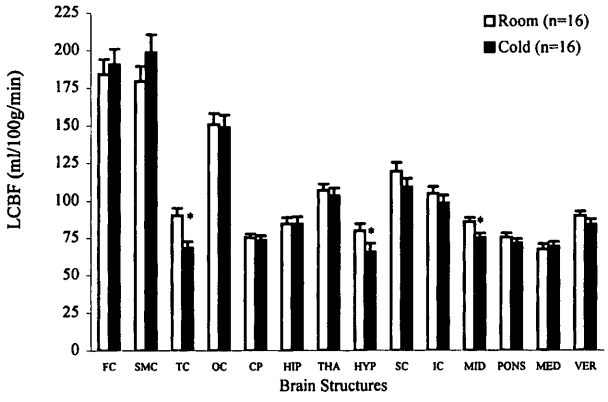

Fig. 1. Local Cerebral Blood Flow (LCBF) (ml/100g/min) of 14 brain structures in room temperature versus cold temperature for the WKY. Abbreviations are as in Table 2. *, significantly different from room group ($p<0.05$, MANOVA followed by univariate F-test).

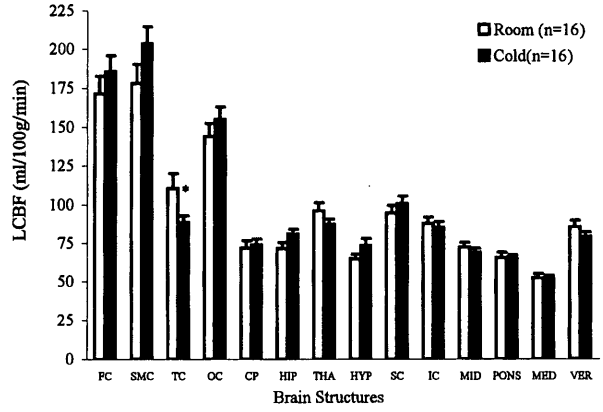

Fig. 2. Local Cerebral Blood Flow (LCBF) (ml/100g/min) of 14 brain structures in room temperature versus cold temperature for the SHR. Abbreviations are as in Table 2. *, significantly different from room group ($p<0.05$, MANOVA followed by univariate F-test).

Discussion

The main finding of this study is that a transient cold exposure (5°C) for 30 min resulted in a significant decrease in LCBFs in TC, HYP, MID of WKY, and TC of SHR. SHR also displayed a distress response, including increases in heart rate and blood glucose, although did not lead to a significant alteration in blood pressure. This cold exposure caused a significant decrease in body temperature for both WKY and SHR. TNx did not alter any physiological parameters in both ambient temperature or cold environment.

The etiology of the increased stroke prevalence in winter is unknown. Several factors have been proposed, including elevated blood pressure, derangement of brain circulation, and rheological factors in winter. Our data show that the exposure to cold did not induce any change of blood pressure and hematocrit. However, LCBFs significantly dropped at the TC of WKY and SHR, and HYP/MID of WKY. These findings indicate that the decreased LCBFs in some brain structures after a transient cold exposure may be partly responsible for the high prevalence of ischemic stroke in winter. However, bleeding in the brain was not found in both WKY and SHR, probably because hypertensive response was not induced. Therefore, the present experiment can not explain the high prevalence of hemorrhagic stroke in human. In this concerns, a stroke-prone SHR which is prone to suffer from hemorrhagic stroke upon stress could be a better animal model for the future study.

The reason why LCBFs at some brain areas decreased after the cold exposure in both SHR and WKY should be explained. Since BP was unchanged by the cold exposure, the decreased LCBFs were not related to BP. However, both WKY and SHR showed significant decreases by 1.3-2.3°C in core body temperature. This might lead to a decrease in brain metabolism and thereby a decrease of LCBFs. The rationale for the cold stimulus (5°C) environment for 30 min is as follows. First, the lowest temperature in Taiwan is about 5°C in the winter. In most time of the winter season, the environmental temperature is around 10-15°C. This mild low ambient temperature is sufficient to increase the stroke prevalence in Taiwan (9). Second, this mild cold stimulation was sufficient to induce a decrease in core body temperature by 1.3-2.3°C (Table 1). If a vigorous cold stimulation is applied, the rats may become difficult to maintain their body temperature and to sustain it through the entire experimental procedure.

Evidence shows that cold receptors exist on the face and nasal mucosa (30) to sense the cold environment. The cold information is then send through the afferent cold fibers of the supra- and infra-orbital nerves, anterior ethmoid and posterior nasal nerves to the trigeminal ganglion (1, 6, 30). Recently, neurons in the trigeminal ganglion have been found to have their axons innervate the cerebral blood vessels. These axons contain substance P and CGRP by which the diameter of cerebral blood vessels is modulated. Moreover, these axons augment LCBFs during acute hypertension and seizure via an axon reflex-like mechanism (22). Other nerves, such as the intracranial branches of the sphenopalatine ganglion, can cause dilatation of the middle cerebral arteries and increase LCBF in the ischemic forebrain in rats (10, 15). These indicate that the trigeminal nerve may have only modulatory action on cerebral blood vessels. On the other hand, several reports (24, 29) demonstrated that TNx did not alter LCBFs in monkeys and rats, indicating the trigeminal nerve may not have

tonic control of the basal CBF. These results were confirmed by the present findings that TNx did not alter LCBFs in WKY and SHR when animals were either kept at room temperature or put in a cold environment. These findings also indicate that cold afferents in the tirgeminal nerve may not participate in modulation of the basal CBFs. Whether more severe cold stress lower than 5°C may modulate the basal CBFs needs further investigation.

In conclusion, the finding that transient exposure to cold leads to decrease body temperature and LCBFs at some parts of brain areas in both normotensive and hypertensive animals may be partially responsible for the high prevalence of stroke in winter. The findings that TNx did not alter LCBFs in WKY and SHR in either room temperature or cold environment may suggest lack of tonic control of the LCBFs by the tirgeminal nerve, as well as lack of modulatory effect on the LCBFs by the cold afferents in the trigeminal nerve.

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