

Role of the Peripheral Sympathetic Innervations in Controlling Cerebral Blood Flow after the Transection of Bilateral Superior Cervical Sympathetic Ganglia Two Weeks Later

Cheng-Ta Hsieh^{1,2}, Shinn-Zong Lin³, Ming-Ying Liu¹

¹*Department of Neurological Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, China*

²*Division of Neurosurgery, Department of Surgery, Sijhih Cathay General Hospital, New Taipei, China*

³*Division of Neurosurgery, Department of Surgery, China Medical University Hospital, Taichung, Taiwan, China*

E-mail: nogor@mail2000.com.tw

Received March 30, 2011; revised May 5, 2011; accepted May 10, 2011.

Abstract

Background: Cerebral blood vessels are mainly supplied by sympathetic nerves arising from the superior cervical ganglia and cerebral blood volume may be influenced by bilateral superior cervical ganglionectomy (SCG). Various stages of cerebral blood volume changes depended on the time following bilateral excision of SCG. In this study, we emphasize the subacute effect (two weeks) on the local cerebral blood flow (LCBF). **Material and Methods:** Sprague-Dawley rats weighing 250 - 400 gm ($n = 20$) were selected into two groups. Under the ambient temperature 20°C, the first group animals ($n = 10$) received sham operation and the other group animals ($n = 10$) underwent bilateral SCG. The LCBF and O₂ delivery of 14 brain structures were measured for each animal by the use of ¹⁴C-iodoantipyrine technique two weeks after the operation. **Results:** The average of LCBF was decreased from 150 ml/100 gm/min to 129 ml/100 gm/min after bilateral SCG. Only the LCBF at basal ganglia was increased from 108 ml/min/100 g in the sham-operated group to 118 ml/min/100g in the SCG group. A mean of 14% reduction of LCBF was estimated. In 14 brain structures, the delivery amount of O₂ was all decreased, except in basal ganglia. However, these changes of LCBF and the delivery amount of O₂ at these 14 brain structures did not reach the significant differences. **Conclusions:** The present results show that chronic effect (two weeks) of bilateral SCG on LCBF was not only in a decrease of the LCBF, but also a decrease of local cerebral O₂ delivery. However, the changes didn't show the significant differences.

Keywords: Local Cerebral Blood Flow, Cervical Ganglia, Ganglionectomy

1. Introduction

The relationship between autonomic regulation and cerebral blood flow (CBF) has long been a subject of considerable interest in studying the mechanisms of neurovascular disorders such as hypertensive encephalopathy, migraine and syncope [1]. Cerebral blood vessels are mainly supplied by sympathetic nerves arising from the superior cervical ganglia, and the sympathetic nerves, heterogeneously distributed throughout the cere-

brocortex [2,3]. Although nervous stimulation or adrenergic agonists lead to vasoconstriction, superior cervical ganglionectomy (SCG) or adrenergic antagonists do not usually affect regional CBF [4,5]. "Microregional" differences in the cerebrocortex without decreasing global and regional blood flow, resulted from limit flow and/or decrease red cell velocity, has been hypothesized [6]. The subacute effect of SCG on cerebral microcirculation remains obscure. In this study, we evaluate local cerebral blood flow (LCBF) and the delivery amount of O₂ at 14

brain structures in experimental rats underwent bilateral SCG.

2. Material and Methods

Male Sprague-Dawley rats weighing 250 to 400 gm were used for these experiments. These animals were fasted for approximately 12 hours before surgery.

2.1. Surgical Techniques

The rats were anesthetized with 3% halothane for 2 - 4 minutes until they were calm down. The anesthesia was continued with 1.5% halothane in nitrous oxide (70%) and oxygen (30%) for the period of surgery. The femoral artery and femoral vein were catheterized on both sides of the animals with polyethylene tube (PE-50). The surgical wounds were covered with xylocaine jelly. A plaster cast was fitted from midthorax to midthigh to immobilize the hind limbs and protect the catheters. Then the anesthesia was stopped and the animals were allowed to recover the consciousness.

2.2. Experimental Design

The rats were exposed in normal condition (ambient temperature 20°C) and their physiological condition was assessed by measurement of hematocrit (Hct), hemoglobin (Hgb), pH, pCO₂, pO₂, volume and saturation of O₂, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MBP), pulse rate (PR), weight, and plasma glucose level. Total 20 male Sprague-Dawley rats were divided into two groups: Group A ($n = 10$) as the control group underwent sham operation. Group B ($n = 10$) received bilateral superior cervical ganglionectomy. Local cerebral blood flow and O₂ delivery were measured post each operation. Superior cervical ganglionectomy was performed as: the salivary glands were exposed through sublingular incision in the neck and each superior cervical ganglion was identified at the bifurcation of the common carotid artery beside the trachea. The ganglia were totally removed en bloc from both sides. Sham operation received the same procedure as above except the removal of SCG. After recovering from the anesthesia, the rats were put in a cage with food as usual. Two weeks later, the rats were re-anesthetized for measurement of LCBF.

2.3. Measurement of Local Cerebral Blood Flow

The LCBF was measured by the ¹⁴C-iodoantipyrine (IAP) technique developed by Sakurada *et al.* [7], as modified by Otsuka *et al.* [8]. Before beginning IAP infusion, a

single extracorporeal arteriovenous (AV) shunt was formed by shortening and connecting the femoral artery and vein catheters on one side with a 3-cm length of silicone rubber tubing. At the beginning of the experimental period ($t = 0$), an intravenous infusion of approximately 50uCi of ¹⁴C-IAP in 1ml saline was started using a variable speed infusion pump. The rate of infusion was increased during the experiment according to a schedule that yielded a linearly rising concentration of IAP in the blood. Series arterial blood sampling was performed (every 5 sec starting at $t = 0$) by puncturing the extracorporeal AV loop with a 22-gauge needle on a plungerless syringe and collecting 60 ~ 80 µl blood over the next 2 - 4 sec. The rats were decapitated at $t = 30$ sec, and their brains were quickly removed, frozen in 2-methylbutane cooled to -45°C, and stored in a plastic bag at -80°C until the time of sectioning.

Plasma ¹⁴C-radioactivity was assayed by beta counting. The frozen brain was cut into a series of 20 µm thick sections in a cryostat at -20°C starting at the area postrema, which lies at the caudal end of the fourth ventricle. Selected brain sections from inferior colliculus, superior colliculus, midbrain, occipital cortex, pons, medulla oblongata, hippocampus, hypothalamus, thalamus, corpus callosum, temporal cortex, frontal cortex, basal ganglia, sensorimotor cortex, cerebellar vermis were dried and placed in X-ray cassettes along with sets of previously calibrated ¹⁴C standards. Many of the areas were selected because they had been included in the reports of Kadekaro *et al.* [9] and our previous study [10]. Un-exposed X-ray film (Kodak SB-5) was placed over the brain section and standards. After approximately 12 days of exposure, the films were developed. The entire autoradiograms were analyzed for radioactivity using an image processing system (MCID, Imaging Research, St. Catharings, Canada). The optical density values were converted to tissue reactivity using the Kety-Sokoloff equation [7].

2.4. Measurement of O₂ Delivery

Adjacent frozen samples from fourteen brain regions were immediately used to determinate arterial and venous O₂ saturation. Detail techniques have been published previously [11]. Briefly, 20 µm sections were obtained on the microtome-cryostat at -35°C under a N₂ atmosphere. The sections were transferred to precooled glass slides and covered with degassed silicone oil and a cover slip. The slides were placed on a microspectrophotometer fitted with an N₂-flushed cold stage to obtain readings of optical densities at 568, 560, and 506 nm. This three-wavelength method corrects for the light scattering in the frozen blood. Only vessels in the transverse

section were studied so that the path of light traversed only the blood. Readings were obtained to determine O₂ saturation in these regions. The O₂ content of blood was determined by multiplying the percent O₂ saturation by the hemoglobin concentration times 1.36.

2.5. Statistics

All data was presented with means \pm SEM and statistically calculated by unpaired, two-tails *t* test.

3. Results

Hemodynamic and physiological data including plasma glucose level, HCT, pH, pulse rate, weight, systolic blood pressure, diastolic blood pressure, mean blood pressure in two groups revealed no significant difference (**Table 1**). According to the relevant data of blood gas, a significant hyperoxemia (pO₂ from 87.1 to 97 torrs, *p* < 0.05) was demonstrated, but the pCO₂, the volume of O₂ and the saturation of O₂ did not reach the significant differences.

LCBF at 14 brain structures was presented in **Table 2**. Only the LCBF at basal ganglia was increased from 108 ml/min/100 g in the SCG group to 118 ml/min/100 g in

the sham-operated group. The others 13 brain structures showed a decreased level of LCBF. The average of LCBF was 150 ml/min/100 g in the sham-operated group and 129 ml/min/100 g in the SCG group. A mean of 14% reduction of LCBF was found at 14 brain structures. However, these changes of LCBF at these 14 brain structures did not reach the significant differences.

The delivery amount of O₂ was also measured at 14 brain structures and summarized in **Table 3**. In 14 brain structures, the delivery amount of O₂ was all decreased, except in basal ganglia. The differences in the delivery amount of O₂ between sham-operated and sympathectomized group was not significantly meaningful in these 14 brain structures.

4. Discussion

Sympathetic nerves supply different parts of the cerebrovascular system including the main pial arteries at the base of the brain, the pial arterial and venous systems of the convexities, and parts of the intracerebral (parenchymal) vascular system [12]. Using the techniques of immunofluorescence and histofluorescence, cerebral vessels have been proved to be innervated by peripheral sympathetic nervous system, mainly arising from the ipsilateral

Table 1. Hemodynamic and physiological data in experimental rats under sham operation and SCG.

	HCT	HgB	pH	pCO ₂	pO ₂	O ₂ vol.	O ₂ sat.
Sham operation	49.91 \pm 0.48	14.93 \pm 0.09	7.41 \pm 0.03	40.67 \pm 1.56	87.1 \pm 0.55	0.201 \pm 0.001	96.47 \pm 0.23
SCG	49.6 \pm 0.93	14.42 \pm 0.27	7.4 \pm 0.02	38 \pm 1.48	97 \pm 3.94*	0.2 \pm 0.001	97.2 \pm 0.37
	SBP	DBP	MBP	PR	Wt	Glucose	
Sham operation	143.33 \pm 2.11	101.67 \pm 4.01	115.56 \pm 3.23	363.33 \pm 17.26	318.67 \pm 20.49	104.17 \pm 24.67	
SCG	139.17 \pm 5.13	104.83 \pm 3.93	116.28 \pm 3.87	365 \pm 16.88	335.33 \pm 23.33	98.67 \pm 5.77	

*: significant difference between sham operation and SCG group in room temperature (two tailed, unpaired-t test), *P* < 0.05, values are means \pm SEM. HCT: hematocrit; HgB: hemoglobin; pH, pCO₂, pO₂: blood gas; O₂ vol.: O₂ volume; O₂ sat.: O₂ saturation; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean arterial blood pressure; PR: pulse rate; Wt: weight.

Table 2. Local cerebral blood flow (ml/min/100gm) at 14 brain structures in rats underwent sham operation and SCG in room temperature.

	Forebrain						
	FC	SMC	TC	OC	BG	HIP	Hypo
Sham operation	234 \pm 38	239 \pm 40	125 \pm 23	190 \pm 27	108 \pm 22	119 \pm 14	151 \pm 15
SCG	191 \pm 15	203 \pm 13	107 \pm 12	182 \pm 17	118 \pm 21	95 \pm 6	133 \pm 9
	Rostral hindbrain			Caudal hindbrain			
	SC	IC	Midbrain	Pons	Medulla	Vermis	
Sham operation	166 \pm 25	155 \pm 30	133 \pm 14	117 \pm 12	102 \pm 10	135 \pm 17	
SCG	126 \pm 8	138 \pm 8	103 \pm 7	117 \pm 27	83 \pm 4	108 \pm 6	

Values are means \pm SEM. FC: frontal cortex; SMC: sensorimotor cortex; TC: temporal cortex; OC: occipital cortex; BG: basal ganglia; HIP: hippocampus; HYPO: hypothalamus; SC: superior colliculus; IC: inferior colliculus.

Table 3. O₂ delivery (ml/min/100gm) at 14 brain structures in rats underwent sham operation and SCG in room temperature.

	Forebrain							
	FC	SMC	TC	OC	BG	HIP	Thalamus	Hypo
Sham operation	47 ± 8	48 ± 8	25 ± 4	38 ± 5	22 ± 4	24 ± 3	30 ± 3	24 ± 3
SCG	38 ± 3	40 ± 2	21 ± 2	36 ± 3	23 ± 4	19 ± 1	26 ± 2	18 ± 2
	Rostral hindbrain				Caudal hindbrain			
	SC	IC	Midbrain	Pons	Medulla	Vermis		
Sham operation	33 ± 5	31 ± 6	27 ± 3	24 ± 2	20 ± 2	27 ± 3		
SCG	25 ± 2	27 ± 2	20 ± 1	23 ± 5	17 ± 1	21 ± 1		

Values are means ± SEM. FC: frontal cortex; SMC: sensorimotor cortex; TC: temporal cortex; OC: occipital cortex; BG: basal ganglia; HIP: hippocampus; HYPO: hypothalamus; SC: superior colliculus; IC: inferior colliculus.

superior cervical ganglion [13]. These nerve fibers are distributed mainly in rostral areas of the brain and heterogeneously distributed to cortical vessels to the level of arterioles [5]. The innervation also provides a protective mechanism for brain to insure an adequate O₂ supply to caudal regions under hypoxic conditions by limiting flow to cortical areas [6].

Several studies about sympathetic influences on cerebral vessels have been discussed, especially in SCG, by measuring the cerebral blood volume (CBV) and regional CBF. Eklof et al. discovered CBF reduced about 30% in monkeys undergo bilateral SCG within 2 weeks but the same results were not observed by Waltz et al in the cat after monolateral SCG [14, 15]. Several experimental studies revealed the various stages of CBV depended on the time following bilateral SCG [12, 16, 17]. In serial mice studies, Edvinsson et al. found, compared with sham-operated controls, CBV was decreased to a level 15% to 28% below the control values twelve hours after SCG, increased to a level 15% to 34% above the control values one day later, and returned to normal range within only one week [12, 16]. He hypothesized the vasoconstriction was caused by norepinephrine leakage from the degenerating nerves shortly after operation; vasodilatation resulted from a consequence of disappearance of the transmitter one day later; and normalization of vascular tone was caused by supersensitivity of denervated vascular receptors to circulating catecholamines in chronic phase [12].

However, in the studies emphasized on the long term effects (8 - 10 weeks) of unilateral SCG on non-anesthetized rabbits, Aubineau *et al.* [18], found a mean of 17% reduction of CBF compared to the heterolateral cortex. This phenomenon was observed from 8 to 30 days after the operation. Intravenous infusion of noradrenaline did not significantly modify CBF in both hemispheres. There were no signs of supersensitivity to catecholamines. They concluded that, as in the peripheral circulation, chronic sympathectomy affects the equilibrium of the

vascular smooth muscle fibers, but that circulating amines play no compensatory role in the cerebral circulation because of the blood-brain barriers. So the reduction of CBF could be attributed to many factors after sympathectomy: decrease in the thickness of the tunica media of the cerebral vessels [19], a modification of membrane mechanisms of the smooth muscle fibers [20], depolarization without any possible modification in the threshold of excitability, a hypersensitivity to calcium ion, and possible metabolic changes revealed by a decrease in sodium-potassium-adenosine triphosphatase activity and modification in the level of adenosine triphosphate [18]. Suppression of the whole cervical sympathetic innervation could also result in a reduction in pineal gland activity [21], alterations in the numerous neuroendocrine systems [22], or a modification in the activity of the carotid sinus [23]. Some of these phenomena may modify CBF and CBV in the long term.

In our present study, two weeks postoperatively, the bilateral SCG rats displayed a mean of 14% reductive level of LCBF as compared to those of the sham-operated rats. In the 14 brain structure, the LCBF was all decreased, except in the basal ganglia. It showed the subacute effect of bilateral SCG was not only in the CBF, but also in the LCBF. The delivery amount of O₂ was also decreased in these regions. Our results were compatible with previous reports [11, 18]. Based on these results, we could conduct the study to investigate the further effect of bilateral SCG in the rats with heat stroke.

5. Conclusions

Although the change of LCBF and local cerebral O₂ delivery in the 14 brain structure didn't show the significant differences, our present results show that subacute effect (two weeks) of bilateral SCG on CBF was not only in a decrease of LCBF, but also a decrease of local cerebral O₂ delivery. About 14% decrease level of LCBF was

observed

6. References

- [1] Y. H. Sohn, "Cerebral Hemodynamic Changes Induced by Sympathetic Stimulation Tests," *Yonsei Medical Journal*, Vol. 39, No. 4, 1998, pp. 322-327.
- [2] D. W. Busija, "Sustained Cerebral Vasoconstriction during Bilateral Sympathetic Stimulation in Anesthetized Rabbits," *Brain Research*, Vol. 345, No. 2, 1985, pp. 341-344. [doi:10.1016/0006-8993\(85\)91013-3](https://doi.org/10.1016/0006-8993(85)91013-3)
- [3] L. Edvinsson, "Neurogenic Mechanisms in the Cerebrovascular Bed. Autonomic Nerves, Amine Receptors and Their Effects on Cerebral Blood Flow," *Acta physiologica Scandinavica. Supplementum*, Vol. 427, 1975, pp. 1-35.
- [4] S. Sadoshima, K. Fujii, K. Kusuda, O. Shiokawa, H. Yao and S. Ibayashi, "Importance of Bilateral Sympathetic Innervation on Cerebral Blood Flow Autoregulation in the Thalamus," *Brain Research*, Vol. 413, No. 2, 1987, pp. 297-301. [doi:10.1016/0006-8993\(87\)91020-1](https://doi.org/10.1016/0006-8993(87)91020-1)
- [5] H. M. Wei, A. K. Sinha and H. R. Weiss, "Cervical Sympathectomy Reduces the Heterogeneity of Oxygen Saturation in Small Cerebrocortical Veins," *Journal of Applied Physiology*, Vol. 74, No. 4, 1993, pp. 1911-1915.
- [6] H. M. Wei, W. Y. Chen, A. K. Sinha and H. R. Weiss, "Effect of Cervical Sympathectomy and Hypoxia on the Heterogeneity of O₂ Saturation of Small Cerebrocortical Veins," *Journal of Cerebral Blood Flow & Metabolism*, Vol. 13, No. 2, 1993, pp. 269-275. [doi:10.1038/jcbfm.1993.33](https://doi.org/10.1038/jcbfm.1993.33)
- [7] O. Sakurada, C. Kennedy, J. Jehle, J. D. Brown, G. L. Carbin and L. Sokoloff, "Measurement of Local Cerebral Blood Flow with Iodo [14C] Antipyrine," *American Journal of Physiology*, Vol. 234, No. 1, 1978, pp. H59-66.
- [8] T. Otsuka, L. Wei, V. R. Acuff, A. Shimizu, K. D. Pettigrew, C. S. Patlak, "Variation in Local Cerebral Blood Flow Response to High-Dose Pentobarbital Sodium in the Rat," *American Journal of Physiology*, Vol. 261, No. 1-2, 1991, pp. H110-120.
- [9] M. Kadekaro, H. E. Savaki, F. A. Kutyna, L. Davidsen, L. Sokoloff, "Metabolic Mapping in the Sympathetic Ganglia and Brain of the Spontaneously Hypertensive Rat," *Journal of Cerebral Blood Flow & Metabolism*, Vol. 3, No. 4, 1983, pp. 460-467. [doi:10.1038/jcbfm.1983.72](https://doi.org/10.1038/jcbfm.1983.72)
- [10] S. Z. Lin, N. Sposito, S. Pettersen, L. Rybacki, E. McKenna, K. Pettigrew, "Cerebral Capillary Bed Structure of Normotensive and Chronically Hypertensive Rats," *Microvascular Research*, Vol. 40, No. 3, 1990, pp. 341-357. [doi:10.1016/0026-2862\(90\)90032-M](https://doi.org/10.1016/0026-2862(90)90032-M)
- [11] M. T. Lin and S. Z. Lin, "Decentralization of Superior Cervical Ganglia Attenuates Heat Stroke Formation in Rabbits," *The Chinese Journal of Physiology*, Vol. 33, No. 3, 1990, pp. 247-253.
- [12] L. Edvinsson, K. C. Nielsen, C. Owman, K. A. West, "Evidence of Vasoconstrictor Sympathetic Nerves in Brain Vessels of Mice," *Neurology*, Vol. 23, No. 1, 1973, pp. 73-77.
- [13] M. E. Raichle, B. K. Hartman, J. O. Eichling and L. G. Sharpe, "Central noradrenergic regulation of cerebral blood flow and vascular permeability," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 72, No. 9, 1975, pp. 3726-3730. [doi:10.1073/pnas.72.9.3726](https://doi.org/10.1073/pnas.72.9.3726)
- [14] B. Eklof, D. H. Ingvar, E. Kagstrom and T. Olin, "Persistence of Cerebral Blood Flow Autoregulation Following Chronic Bilateral Cervical Sympathectomy in the Monkey," *Acta Physiologica Scandinavica*, Vol. 82, No. 2, 1971, pp. 172-176. [doi:10.1111/j.1748-1716.1971.tb04956.x](https://doi.org/10.1111/j.1748-1716.1971.tb04956.x)
- [15] A. G. Waltz, T. Yamaguchi and F. Regli, "Regulatory Responses of Cerebral Vasculature after Sympathetic Denervation," *American Journal of Physiology*, Vol. 221, No. 1, 1971, pp. 298-302.
- [16] L. Edvinsson, K. C. Nielsen, C. Owman, K. A. West, "Sympathetic Adrenergic Influence on Brain Vessels as Studied by Changes in Cerebral Blood Volume of Mice," *European Neurology*, Vol. 6, No. 1, 1971, pp. 193-202. [doi:10.1159/00014492](https://doi.org/10.1159/00014492)
- [17] S. H. Tsai, S. Z. Lin and C. J. Shih, "Effects of Pre-Ganglionic Decentralization or Post-Ganglionic Excision of the Superior Cervical Ganglia on Brain Edema and Heat Stroke in Rats," *Proceedings of the National Science Council, Republic of China. Part B*, Vol. 8, No. 4, 1984, pp. 335-340.
- [18] P. Aubineau, A. M. Reynier-Rebuffel, C. Bouchaud, O. Jousseau, J. Seylaz, "Long-Term Effects of Superior Cervical Ganglionectomy on Cortical Blood Flow of Non-Anesthetized Rabbits in Resting and Hypertensive Conditions," *Brain Research*, Vol. 338, No. 1, 1985, pp. 13-23. [doi:10.1016/0006-8993\(85\)90243-4](https://doi.org/10.1016/0006-8993(85)90243-4)
- [19] R. D. Bevan, H. Tsuru and J. A. Bevan, "Cerebral Artery Mass in the Rabbit is Reduced by Chronic Sympathetic Denervation," *Stroke*, Vol. 14, No. 3, 1983, pp. 393-396. [doi:10.1161/01.STR.14.3.393](https://doi.org/10.1161/01.STR.14.3.393)
- [20] O. Aprigliano, "Neural Influences and Norepinephrine Sensitivity in the Rat Portal Vein," *Fed Proc*, Vol. 42, No. 2, 1983, pp. 257-262.
- [21] M. A. Luchelli-Fortis, F. J. Stefano and C. J. Perc, "Degeneration Activity of the Pineal Gland after Sympathetic Denervation," *Naunyn-Schmiedeberg's Archives of Pharmacology*, Vol. 321, No. 4, 1982, pp. 298-301. [doi:10.1007/BF00498517](https://doi.org/10.1007/BF00498517)
- [22] D. P. Cardinali, M. I. Vacas, P. V. Gejman, M. A. Pisarev, M. Barontini and R. J. Boado, "The Sympathetic Superior Cervical Ganglia as 'Little Neuroendocrine Brains'," *Acta physiologica latino americana*, Vol. 33, No. 3, 1983, pp. 205-221.
- [23] K. Koizumi and A. Sato, "Influence of Sympathetic Innervation on Carotid Sinus Baroreceptor Activity," *American Journal of Physiology*, Vol. 216, No. 2, 1969, pp. 321-329.