Published in final edited form as:

Auton Neurosci. 2009 May 11; 147(1-2): 101–104. doi:10.1016/j.autneu.2009.01.009.

Parasympathetic Tonic Dilatory Influences on Cerebral Vessels

Nicholas C. Boysen, Deidre Nitschke Dragon, and William T. Talman, MD

Laboratory of Neurobiology, Department of Neurology, Department of Veterans Affairs Medical Center and Carver College of Medicine, University of Iowa, IowaCity, IA 52242

Abstract

Parasympathetic nerves from the pterygopalatine ganglia may participate in development of cluster headaches, in vascular responses to hypertension and in modulation of damage due to stroke. Stimulation of the nerves elicits cerebral vasodilatation, but it is not known if the nerves tonically influence cerebrovascular tone. We hypothesized that parasympathetics provide a tonic vasodilator influence and tested that hypothesis by measuring cerebral blood flow in anesthetized rats before and after removal of a pterygopalatine ganglion. Ganglion removal led to reduced cerebral blood flow without changing blood pressure. Thus, parasympathetic nerves provide tonic vasodilatory input to cerebral blood vessels.

Keywords

Cerebral blood flow; Parasympathetic; Vasodilatation

Cerebral blood vessels are richly innervated both by central pathways (Reis, 1984;Vaucher & Hamel, 1995) and by sympathetic and parasympathetic nerves (Wahl & Schilling, 1993) and are further influenced by metabolic activity of local CNS neurons (Dirnagl *et al.*, 1994;Harder *et al.*, 2002). We have focused on the parasympathetic innervation derived from neurons of the pterygopalatine ganglion (PPG), also referred to as the “sphenopalatine ganglion”. We, like others (Morita-Tsuzuki *et al.*, 1993), have shown that electrical stimulation of the PPG causes cerebral vasodilatation (Talman *et al.*, 2007). Furthermore, we have shown that the PPG is critical for expression of cerebral vasodilatation mediated by hypertension (Talman & Nitschke Dragon, 2000), that the dilatation is dependent on arterial baroreflexes (Talman *et al.*, 1994), and that those dilatory influences are themselves mediated by nitric oxide synthesized by local neurons (Talman & Nitschke Dragon, 2004;Talman & Nitschke Dragon, 2007). One published study (Toda *et al.*, 2000) directly tested whether parasympathetic input to cerebral arteries may contribute tonic vasodilatory influences to the tone in those vessels and another tested that possibility in ophthalmic arteries (Ayajiki *et al.*, 2000). In both studies, however, cerebral blood flow (CBF) and cerebral vascular resistance were assessed by measuring pial arterial diameter and concomitant blood pressure and not by a direct measurement of flow. Furthermore, the study of cerebral vessels after removal of the PPG was performed in dogs (Toda *et al.*, 2000). In contrast, some studies in which influences on cerebrovascular tone were assessed after interruption of parasympathetic input to the vessels reported no change in CBF. (Tanaka *et*

Corresponding Author: William T. Talman, MD, Department of Neurology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, e-mail: william-talman@uiowa.edu, Tel. #: 319-356-8750, FAX: 319-384-6269, url: <http://www.uihealthcare.com/depts/med/neurology/neurologymds/talman.html>.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

et al., 1995b; Branston *et al.*, 1995). In that varying influences on CBF also have been appreciated in different species with stimulation of parasympathetic nerves (Busija & Heistad, 1981; Morita-Tsuzuki *et al.*, 1993; Talman *et al.*, 2007), in that inhibition of nitric oxide synthase attenuates the cerebral blood flow response to stimulation of postganglionic parasympathetic nerves in the rat (Morita-Tsuzuki *et al.*, 1993) and in that much of our own work focuses on rat models of cerebrovascular disease, here we tested the hypothesis that parasympathetic innervation provides a tonic vasodilatory influence on cerebral blood vessels in rat. We used laser flowmetry to assess changes in CBF in the parietal lobe of the brain in rats after acute removal of the ipsilateral pterygopalatine ganglion.

All protocols were approved by the institutional animal care and use committees of the University of Iowa and the Department of Veterans Affairs Medical Center, Iowa City and adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Adult male Sprague Dawley rats (N= 10) were anesthetized with isoflurane (5% induction and 2-2.5% maintenance) delivered in 100% O₂ by nasal cannula to the freely breathing animals. A temperature probe connected to a heating pad was used to maintain body temperature (37°) throughout surgery by means of a temperature controller (YSI Model 73A, Yellow Springs, OH). A cannula (polyethylene, PE-50 tubing) filled with heparinized saline and attached to a transducer and data acquisition system (Power Lab 8/SP; ADInstruments, Colorado Springs) was inserted into a femoral artery for recording arterial blood pressure (AP). The left PPG was exposed with a modification of the method described by Rosen *et al* (Rosen *et al.*, 1940). The rat's mouth was kept open with a 1 cm roll of damp cotton gauze inserted between the upper and lower incisors. Doing so moved the temporal muscle posteriorly to aid in exposing the ganglion. The rat's eye was covered with Triple Antibiotic Ointment (Phoenix Pharmaceutical, St. Joseph, MO). After shaving and preparing the skin with iodine and alcohol we made a 1-1.5 cm incision immediately inferior to the zygomatic arch, exposed the zygomatic bone through blunt dissection, and removed a 1-1.5 cm length of the zygomatic bone with rongeurs. The masseter muscle was then cut and retracted ventrally, to expose the lacrimal gland, which was then retracted dorsally. The maxillary nerve was identified through a microscope, dissected free and retracted dorsally. Using the bony ridge along which the maxillary nerve runs as a guide, the vidian nerve was exposed and the PPG which the nerve enters was isolated. The animal was then placed prone in a stereotaxic frame (DKI Model 1404, David Kopf Instruments, Tujunga, CA). After preparation of the scalp and exposure of the skull a burr hole was made over the parietal lobe ipsilateral to the exposed PPG. The dura was left intact and mineral oil was dripped on it to prevent drying. A laser probe (0.8 mm) mounted in a stereotaxic instrument holder was carefully placed into the mineral oil pool for continuous monitoring of cerebral blood flow (CBF) by a Laserflo Blood Perfusion Monitor (TSI, Model 403A, St. Paul) laser flow meter. Throughout recording of CBF ambient lighting was kept constant. We optimized visualizing the PPG by angling the stereotaxic frame so that the contralateral eye was dependent. As a result, CBF could only be recorded from the cortex ipsilateral to the previously isolated ganglion. The ganglion was again exposed after retraction of surrounding soft tissues. Baseline CBF was established for at least 5 minutes before proceeding with removal of the PPG. Thus, each animal served as its own control. Because we found that touching the ganglion and bony tissues surrounding it often led to artifactual transient increases in CBF with prompt return to basal values, data were collected at baseline before transection and again immediately after transection. Immediately before the resection and during a stable baseline CBF, a sample (0.2-0.25 ml) of arterial blood was removed for blood-gas analysis. All animals freely breathed without ventilatory support throughout the experiments. In that we anticipated that removal of the PPG would lead to a reduction in CBF we chose to accept data if the arterial pCO₂ increased after removal of the ganglion (thus attenuating any decrease in flow that might be observed and biasing against our hypothesis). In that pCO₂ never fell as a result of ganglionectomy, no parameters were set for such relative hypocarbia. After establishing basal CBF and blood gas values we quickly removed the PPG

after cutting its efferent fibers and the vidian nerve as it entered the ganglion. After CBF had achieved a new baseline (typically within 10 minutes of resection) arterial blood was again removed for blood gas analysis.

Data are expressed as mean $\pm$ SEM. CBF measurements are expressed in Laser Doppler (LD) units, a relative quantification of flow. Because of differing basal LD values, changes in CBF were expressed as a percent change from baseline. Data were analyzed by Wilcoxon's sign test and significance was accepted at a p value ≤ 0.05 .

Removal of the PPG caused a gradual 27.9 ± 7.8 % decrease in CBF (decrease of 13.5 ± 6.5 laser units; $p = 0.047$) without changing mean arterial pressure (Fig. 1 and Table 1). Of the 10 animals studied, 9 demonstrated decreased CBF with decreases ranging from 4 LD units (13% fall) to 55 LD units (55% fall). One animal demonstrated a 14 LD unit (18%) increase in CBF after resection. Cerebrovascular resistance, calculated as (mean arterial pressure) $\div$ (CBF in LD units), increased (Table 1) after removal of the ganglion from 1.8 ± 0.3 to 2.9 ± 0.5 resistance units ($p = 0.007$). After removal of the PPG, arterial $p\text{CO}_2$ increased from 26.7 ± 3.2 Torr to 40.1 ± 3.7 Torr ($p < 0.005$) though there was no appreciable change in any animal's breathing. Throughout the study, arterial $p\text{O}_2$ remained greater than 200 Torr in all animals.

This study demonstrates acute vasoconstriction after removal of parasympathetic input to forebrain cerebral arteries and supports a tonic vasodilatory influence by that neural input to the cerebral vasculature. We acknowledge that our study, which did not prohibit an increase in arterial $p\text{CO}_2$, likely underestimated that tonic influence. The data are consistent with studies of cerebrovascular regulation in dogs (Toda *et al.*, 2000) but conflict with other studies in rats (Tanaka *et al.*, 1995a). However, in contrast to the latter study that evaluated CBF two weeks after removal of the parasympathetic innervation, the current study evaluated CBF on-line prior to and immediately after transection. Furthermore, the earlier study utilized autoradiographic analysis of CBF and, thus, was limited to one evaluation of CBF. Therefore, it compared CBF in control animals with that in different animals that had lesions. In contrast, the current study utilized intra-animal controls. We cannot say from our work whether vasoconstriction was transient and would have cleared within two weeks or if the differences in the two studies reflected use of those different methods for CBF analysis. In that parasympathetic innervation of cerebral vessels is predominantly ipsilateral it is unlikely that any changes in CBF would have been found in the contralateral hemisphere had our methods allowed simultaneous bilateral evaluation of flow. However, again studies vary in their reports of contralateral effects of parasympathetic stimulation with some showing such effects (Seylaz *et al.*, 1988) and others finding no such effects (Toda *et al.*, 2000).

At the moment of ganglionectomy in the current study no nerve fibers other than parasympathetic fibers were transected. It then is unlikely that vasoconstriction seen in this study resulted from an influence of damage to any other nerve inputs to the cerebral vasculature. Vasodilatory trigeminal nerve fibers are quite removed from those of the pterygopalatine ganglion and sympathetic fibers, also distant from the parasympathetic fibers, have further been shown to have little or no influence on CBF in the normotensive range of blood pressures seen in this study (Kelly *et al.*, 1994; Sadoshima *et al.*, 1985; Edvinsson *et al.*, 1993; Heistad *et al.*, 1980).

In addition to vasoconstriction, removal of the PPG led to relative hypercarbia, which would attenuate the vasoconstriction seen here. We have no explanation for the increases in $p\text{CO}_2$ that we found. While one study (Hadziefendic & Haxhiu, 1999) suggests that parasympathetic pathways may project to central sites, such as the retrotrapezoid nucleus, (Guyenet, 2008) that are critical for modulating CO_2 , we know of no study that directly demonstrates such a link.

The current study supports a tonic dilatory influence by parasympathetic nerves on cerebral vessels, but the physiological relevance of that influence remains to be determined. Other studies have suggested physiological implications of the innervation. For example, projections from PPG may participate in linking flow with local neuronal activity (Umemura & Branston, 1995). Other studies have suggested that parasympathetic nerves may provide protection to the brain when exposed to ischemia (Kano *et al.*, 1991; Koketsu *et al.*, 1992; Henninger & Fisher, 2007; Solberg *et al.*, 2008) and we have shown a contribution to vasodilatation during acute hypertension (Agassandian *et al.*, 2003; Talman & Nitschke Dragon, 2000). We have conjectured that parasympathetics to cerebral vessels may also contribute to thermoregulation (Agassandian *et al.*, 2003) by diverting blood flow to the brain while, at the same time, promoting salivation and nasal secretion (Loewy & Spyer, 1990), each a contributor to thermoregulation, especially in rodent species (Stricker, 1970). Parasympathetic influences on cerebral blood vessels may not only play a role in normal physiological function but also may contribute to pathological states. For example, parasympathetic nerves from the PPG may participate in development of cluster headaches, which may respond to blocking function of those same nerves (Kittelle *et al.*, 1985; Torelli & Manzoni, 2004). The current study suggests that the beneficial effects from blocking those nerves may be mediated through removal of their tonic vasodilatory influences.

Acknowledgments

This work was supported by VA Merit Review (WTT PI) and in part by NIH R01 HL59593 (WTT PI).

References

- Agassandian K, Fazan VPS, Margaryan N, Nitschke Dragon D, Riley J, Talman WT. A novel central pathway links arterial baroreceptors and pontine parasympathetic neurons in cerebrovascular control. *Cell Molec Neurobiol* 2003;23:463–478. [PubMed: 14514008]
- Ayajiki K, Tanaka T, Okamura T, Toda N. Evidence for nitroxidergic innervation in monkey ophthalmic arteries in vivo and in vitro. *Am J Physiol Heart Circ Physiol* 2000;279:H2006–H2012. [PubMed: 11009491]
- Branston NM, Umemura A, Koshy A. Contribution of cerebrovascular parasympathetic and sensory innervation to the short-term control of blood flow in rat cerebral cortex. *J Cereb Blood Flow Metab* 1995;15:525–531. [PubMed: 7714011]
- Busija DW, Heistad DD. Effects of cholinergic nerves on cerebral blood flow in cats. *Circ Res* 1981;48:62–69. [PubMed: 6777068]
- Dirnagl U, Niwa K, Lindauer U, Villringer A. Coupling of cerebral blood flow to neuronal activation: Role of adenosine and nitric oxide. *Am J Physiol* 1994;267:H296–H301. [PubMed: 8048594]
- Edvinsson, L.; MacKenzie, ET.; McCulloch, J. Cerebral blood flow and metabolism. Raven Press; New York: 1993.
- Guyenet PG. The 2008 Carl Ludwig Lecture: Retrotrapezoid nucleus, CO₂ homeostasis and breathing automaticity. *J Appl Physiol* 2008;105:404–416. [PubMed: 18535135]
- Hadziefendic S, Haxhiu MA. CNS innervation of vagal preganglionic neurons controlling peripheral airways: a transneuronal labeling study using pseudorabies virus. *J Auton Nerv Syst* 1999;76:135–145. [PubMed: 10412837]
- Harder DR, Zhang C, Gebremedhin D. Astrocytes function in matching blood flow to metabolic activity. *News Physiol Sci* 2002;17:27–31. [PubMed: 11821533]
- Heistad, DD.; Gross, PM.; Busija, DW.; Marcus, ML. Cerebral vascular response to loading and unloading of arterial baroreceptors. In: Sleight, P., editor. Arterial baroreceptors and hypertension. Oxford University Press; Oxford: 1980. p. 210-217.
- Henninger N, Fisher M. Stimulating circle of Willis nerve fibers preserves the diffusion-perfusion mismatch in experimental stroke. *Stroke* 2007;38:2779–2786. [PubMed: 17761922]

- Kano M, Moskowitz MA, Yokota M. Parasympathetic denervation of rat pial vessels significantly increases infarction volume following middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 1991;11:628–637. [PubMed: 2050751]
- Kelly PAT, Thomas CL, Ritchie IM. Sympathetic denervation and the cerebrovascular response to hypertension induced by N^G-nitro-L-arginine methyl ester. *Brain Res* 1994;639:309–312. [PubMed: 8205483]
- Kittrelle JP, Grouse DS, Seybold ME. Cluster headache. Local anesthetic abortive agents. *Arch Neurol* 1985;42:496–498. [PubMed: 3994568]
- Koketsu N, Moskowitz MA, Kontos HA, Yokota M, Shimizu T. Chronic parasympathetic sectioning decreases regional cerebral blood flow during hemorrhagic hypotension and increases infarct size after middle cerebral artery occlusion in spontaneously hypertensive rats. *J Cereb Blood Flow Metab* 1992;12:613–620. [PubMed: 1618940]
- Loewy, AD.; Spyer, KM. Central regulation of autonomic functions. Oxford, New York: 1990.
- Morita-Tsuzuki Y, Hardebo JE, Bouskela E. Inhibition of nitric oxide synthase attenuates the cerebral blood flow response to stimulation of postganglionic parasympathetic nerves in the rat. *J Cereb Blood Flow Metab* 1993;13:993–997. [PubMed: 7691856]
- National Research Council. Guide for the care and use of laboratory animals. National Academy Press; Washington, DC: 1996.
- Reis, DJ. Central neural control of cerebral circulation and metabolism. In: MacKenzie, ET.; Seylaz, J.; Bes, A., editors. *LERS*. Vol. 2. Raven Press; New York: 1984. p. 91-119.
- Rosen S, Shelesnyak MC, Zacharias LR. Naso-genital relationship. II Pseudopregnancy following extirpation of the sphenopalatine ganglion in the rat. *Endocrinology* 1940;27:463–468.
- Sadoshima S, Fujishima M, Yoshida F, Ibayashi S, Shiokawa O, Omae T. Cerebral autoregulation in young spontaneously hypertensive rats: effect of sympathetic denervation. *Hypertension* 1985;7:392–397. [PubMed: 3997222]
- Seylaz J, Hara H, Pinard E, Mraovitch S, MacKenzie ET, Edvinsson L. Effect of stimulation of the sphenopalatine ganglion on cortical blood flow in the rat. *J Cereb Blood Flow Metab* 1988;8:875–878. [PubMed: 3192652]
- Solberg Y, Yarnitsky D, Borenstein N, Tanne D, Dayan A, Weiss S, Fisher M. Effectiveness of Sphenopalatine Ganglion Stimulation Therapy in Focal Ischemic Stroke Models: a 24-hour Post-Stroke Window of Treatment. *Stroke* 2008;39(2):665.
- Stricker, EM. Salivary cooling by rats in the heat. In: Hardy, JD.; Gagge, AP.; Stolwuk, JAJ., editors. *Physiological and behavioral temperature regulation*. Charles C Thomas; Springfield: 1970. p. 611-626.
- Talman WT, Corr J, Nitschke DD, Wang D. Parasympathetic stimulation elicits cerebral vasodilatation in rat. *Auton Neurosci* 2007;133:153–157. [PubMed: 17275420]
- Talman WT, Nitschke Dragon D. Parasympathetic nerves influence cerebral blood flow during hypertension in rat. *Brain Res* 2000;873:145–148. [PubMed: 10915822]
- Talman WT, Nitschke Dragon D. Transmission of arterial baroreflex signals depends on neuronal nitric oxide synthase. *Hypertension* 2004;43:820–824. [PubMed: 14981065]
- Talman WT, Nitschke Dragon D. Selective Neuronal Nitric Oxide Synthase Inhibition Attenuates Breakthrough of Autoregulation during Acute Hypertension. *Brain Res* 2007;1139:126–132. [PubMed: 17291465]
- Talman WT, Nitschke Dragon D, Ohta H. Baroreflexes influence autoregulation of cerebral blood flow during hypertension. *Am J Physiol Heart Circ Physiol* 1994;267:H1183–H1189.
- Tanaka K, Fukuuchi Y, Shirai T, Nogawa S, Nozaki H, Nagata E, Kondo T, Suzuki N, Shimizu T. Chronic transection of post-ganglionic parasympathetic and nasociliary nerves does not affect local cerebral blood flow in the rat. *J Auton Nerv Syst* 1995a;53:95–102. [PubMed: 7560762]
- Tanaka K, Fukuuchi Y, Shirai T, Nogawa S, Nozaki H, Nagata E, Kondo T, Suzuki N, Shimizu T. Chronic transection of post-ganglionic parasympathetic and nasociliary nerves does not affect local cerebral blood flow in the rat. *J Auton Nerv Syst* 1995b;53:95–102. [PubMed: 7560762]
- Toda N, Ayajiki K, Tanaka T, Okamura T. Preganglionic and postganglionic neurons responsible for cerebral vasodilation mediated by nitric oxide in anesthetized dogs. *J Cereb Blood Flow Metab* 2000;20:700–708. [PubMed: 10779014]

- Torelli P, Manzoni GC. Cluster headache: symptomatic treatment. *Neurol Sci* 2004;25(Suppl 3):S119–S122. [PubMed: 15549518]
- Umemura A, Branston NM. Cerebrovascular parasympathetic innervation contributes to coupling of neuronal activation and blood flow in rat somatosensory cortex. *Neurosci Lett* 1995;193:193–196. [PubMed: 7478181]
- Vaucher E, Hamel E. Cholinergic basal forebrain neurons project to cortical microvessels in the rat: Electron microscopic study with anterogradely transported *Phaseolus vulgaris* leucoagglutinin and choline acetyltransferase immunocytochemistry. *J Neurosci* 1995;15:7427–7441. [PubMed: 7472495]
- Wahl M, Schilling L. Regulation of cerebral blood flow--a brief review. *Acta Neurochir Suppl (Wien)* 1993;59:3–10. [PubMed: 7508677]

Fig. 1.

In this representative animal arterial pressure (AP), mean AP (MAP), and heart rate (HR) did not change from the basal state prior to removal of the PPG (left) when compared with a period approximately 5 minutes after removal of the left PPG (right). Cerebral blood flow (CBF) did fall after removal of the ganglion ipsilateral to the ganglionectomy. Note: CBF was recorded on a strip chart generated from a laser flowmeter (Laserflo BPM 403A, TSI, St. Paul, MN) while AP and HR were recorded and saved electronically on a Power Lab system (Model 16sp, ADInstruments, Colorado Springs, CO). The two were synchronized post hoc for purposes of displaying all data in this image.

Table 1
Cerebral Vasodilatation after Removal of Pterygopalatine Ganglion

	CBF	MAP	CVR	Arterial pCO ₂
Before PPG Removal	47.5 ± 8.3 LDU	69.1 ± 3.2 mmHg	1.8 ± 0.3 mmHg/LDU	26.7 ± 3.2 Torr
After PPG Removal	34.0 ± 7.7 LDU [*]	70.0 ± 2.9 mmHg	2.9 ± 0.5 [†] mmHg/LDU	36.4 ± 3.7 ^{††} Torr
Change	-27.9 ± 7.8%	0.8 ± 0.6%	59.5 ± 20.0%	13.4 ± 2.0 Torr

^{*} LDU – laser Doppler units; p = 0.047

[†]
p = 0.007

^{††}
p = 0.005

Arterial pO₂ was maintained greater than 200 Torr at all times.