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Short communication

Parasympathetic tonic dilatory influences on cerebral vessels

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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.

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Cerebral blood vessels are richly innervated both by central pathways (Reis, 1984; Vaucher and Hamel, 1995) and by sympathetic and parasympathetic nerves (Wahl and 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 and 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 and Nitschke Dragon, 2004; Talman and 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 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 and 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% O_2 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.

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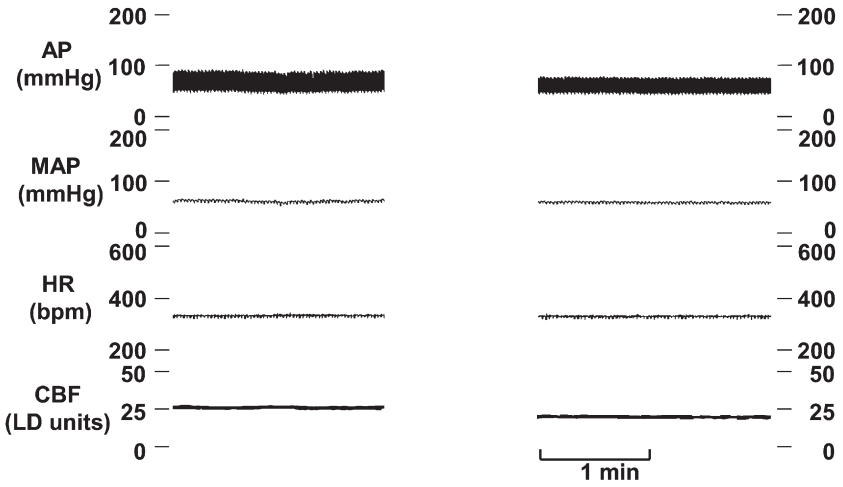

Fig. 1. In this representative animal arterial pressure (AP), mean AP (MAP), and heart rate (HR) did not change from the basal state prior to removal of the PPG (left) when compared with a period approximately 5 min after removal of the left PPG (right). Cerebral blood flow (CBF) did fall after removal 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.

(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 min 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 pCO_2 increased after removal of the ganglion (thus attenuating any decrease in flow that might be observed and biasing against our hypothesis). In that pCO_2 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 min of resection) arterial blood was again removed for blood gas analysis.

Data are expressed as mean ± 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 Wilcoxan'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) ÷ (CBF in LD units), increased (Table 1) after removal of the ganglion from 1.8 ± 0.3 to 2.9 ± 0.5

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

Arterial pO_2 was maintained greater than 200 Torr at all times.
* LDU—laser Doppler units; $p = 0.047$.
† $p = 0.007$.
†† $p = 0.005$.