

Odorants Presented to the Rat Nasal Cavity Increase Cortical Blood Flow

David A. Major and Wayne L. Silver

Department of Biology, Wake Forest University, Winston-Salem, NC 27109, USA

Correspondence to be sent to: Wayne L. Silver, Department of Biology, Wake Forest University, Post Office Box 7325, Winston-Salem NC 27109, USA. e-mail: silver@wfu.edu

Abstract

Complaints about unpleasant environmental odorants, both outdoor and indoor, are increasingly being reported. The main complaints of health symptoms from environmental odorants are eye, nose and throat irritation, headache and drowsiness. Complaints may arise from the stimulation of olfactory receptors or trigeminal chemoreceptors. Stimulation of cerebrovascular nociceptors originating from a branch of the trigeminal nerve may be associated with an increase in cortical blood flow which is thought to be related to headache. Since odorants are reported to elicit headaches, the possibility that odorants may increase cortical blood flow was examined. Cortical blood flow was monitored in rats using a laser-Doppler flowmeter. The flowmeter probe was placed over the left frontal cortex while propionic acid, cyclohexanone, amyl acetate or butanol was delivered to the nasal cavity via an olfactometer. Cortical blood flow increased as the concentration increased for three of the odorants tested. The greatest increase in blood flow occurred to the presentation of propionic acid, followed by cyclohexanone and amyl acetate. There was no response to butanol. These data demonstrate that odorants can alter cerebrovascular blood flow, which may account, in part, for one of the health symptoms reported for odorants.

Introduction

Odorants entering the nasal cavity can stimulate olfactory and/or trigeminal receptors (Silver, 1992). Chemical stimulation of trigeminal fibers innervating the nasal cavity may lead to the perception of irritation and produce local as well as systemic responses. These responses tend to protect the animal from the noxious stimulus and are mediated, in part, by the release (via axon-reflex) of neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) (Maggi and Meli, 1988).

Trigeminal fibers containing SP and CGRP also innervate vascular structures within the cranium, including the meningeal arteries and the large arteries forming the circle of Willis (Saito *et al.*, 1987; Tsai *et al.*, 1988). These vessels are the only pain sensitive structures within the cranium (Ray and Wolff, 1940) and are collectively referred to as the trigeminovascular system. It is now generally believed that stimulation of the trigeminovascular system is responsible for the pain associated with vascular headaches (Moskowitz, 1991; Silberstein, 1992).

Odorants are known to play a role in headaches, such as the precipitation of migraines (Blau and Solomon, 1985). Odorants have also been implicated in what has been described as the 'sick building syndrome', one symptom of which is headache (Hudnell *et al.*, 1992).

Electrical stimulation of trigeminovascular fibers results in an increase in cerebral blood flow (Suzuki *et al.*, 1990a). Sensory trigeminal fibers are sensitive to capsaicin, which,

when presented to the nasal cavity, also alters cerebral blood flow and decreases the frequency of cluster headaches (Fusco *et al.*, 1994). Since a variety of odorants are known to stimulate trigeminal sensory nerve fibers (Silver, 1992), the present study was undertaken to determine whether the presentation of odorants to the nasal cavity of rats would increase cerebral blood flow. If odorants can alter cerebrovascular blood flow, this may account, in part, for one of the health symptoms reported for these compounds.

Materials and methods

Animals and experimental preparation

Ten male Sprague–Dawley rats weighing between 390 and 750 g were used. Each rat was anesthetized with urethane (ethyl carbamate, 1.0 g/kg injected i.p.). Two cannulae were inserted into the trachea of each rat. One cannula was connected to a ventilator and inserted toward the rat's lungs. The rat was artificially ventilated to eliminate breath holding. This was necessary because, as the rat holds its breath, arterial CO₂ increases resulting in increased cortical blood flow (Nakashima *et al.*, 1995). A second cannula, connected to a vacuum line, was inserted up into the nasopharynx. This second cannula was used to draw air/stimuli through the nasal cavity at 250 ml/min. Each rat was then restrained in a head holder. Respiration rate was monitored by placing a rubber strap, connected to a transducer arm, around the rat's torso. Heart rate was monitored by placing

pin electrodes in the left and right pectoral muscles of the rat. Appropriate amplifiers were used to amplify respiration and heart rate signals which were then led to an MP100 data acquisition system running AcqKnowledge software (BIOPAC systems, Goleta, CA) on a Macintosh computer.

A burr hole ~3 mm in diameter was made over the frontal cortex, ~2 mm rostral to the coronal suture and 2 mm to the left of midline. This was performed using a drill under a dissecting microscope. In addition, four of the ten rats had blood flow recorded over the left occipital cortex. This second burr hole was located ~2 mm to the left of the sagittal suture and 1 mm rostral to the interparietal-parietal suture. Since opening the dura mater causes the loss of cerebrovascular autoregulation (Morita *et al.*, 1995), the innermost transparent layer of bone was left intact.

Blood flow measurements

Blood flow was recorded using a Moor dual probe laser-Doppler blood flow monitor (MBF3D). Laser-Doppler flowmetry provides a convenient, non-invasive method for recording relative blood flow changes. The probes were positioned using a micromanipulator so that the tip rested on the bone at the bottom of the burr hole. Since blood flow in the bone is negligible and flow in the dura is small, the registered blood flow represents mainly cortical flow. Mineral oil was added to the burr hole to prevent desiccation. The principles of laser-Doppler flowmetry are described elsewhere (Skarphedinsson *et al.*, 1988).

The laser-Doppler flowmeter calculates the volume and velocity of moving particles within the recording area. These two values are multiplied together to give an arbitrary flux value. Flux was recorded using the MP100 acquisition system and AcqKnowledge software.

Odorant presentation

Odorants were presented using a computer-controlled, air-dilution olfactometer. The design and operation of the olfactometer have been thoroughly described by Silver *et al.* (Silver *et al.*, 1990). Briefly, the apparatus works by mixing a clean air stream and an air stream saturated with an odorant. By combining the two air streams in different proportions, different concentrations of odorants can be presented. The two air streams combine for a final flow rate of 2 l/min. The odorants used were propionic acid (1242, 392 and 124 ppm), cyclohexanone (1416, 448 and 142 ppm), amyl acetate (3020, 955 and 302 ppm) and butanol (6546, 1306 and 260 ppm). These odorants, at the concentrations used, have been shown previously to stimulate the ethmoid branch of the trigeminal nerve (Silver, 1992). All concentrations of each odorant were presented to all ten rats. Stimulus duration was 90 s. Clean air was presented as a control. Each odorant was presented in a series of increasing concentrations while the order of the odorants used was randomized for each rat.

Data analysis

Cortical blood flow, respiration rate and heart rate were recorded for 90 s before, during and after odorant presentation. For each trial, the blood flow values recorded during the 90 s prior to odorant presentation were averaged from 13 500 data points. This mean then served as a baseline for the entire trial (270 s). The blood flow for each 10 s block during a trial was recorded as a percentage change from this averaged baseline. Blood flow, respiration rate and heart rate were then analyzed using a 4 (concentration) \times 18 (time) repeated measures analysis of variance. Significance was examined using Tukey's comparison of means at $P < 0.05$.

To determine if the cortical blood flow during an individual trial increased significantly above controls, the mean blood flow for all clean air trials was calculated to be -0.74% (± 2.12 SEM). A significant increase for an individual trial was then set at two standard deviations ($+8.96\%$) above the calculated control mean.

Results

Three of the four odorants presented to the rats resulted in a significant increase in cortical blood flow. Propionic acid at 1242 and 392 ppm caused a significant increase in blood flow above that recorded during the clean air trials (Figure 1). These values reached significance ~50 s after stimulus onset for 1242 ppm and 60 s after stimulus onset for 392 ppm (Figure 1). Cortical blood flow remained significantly higher than controls for the remainder of the trial (Figure 1). The lowest concentration of propionic acid (124 ppm) did not result in a significant increase in cortical blood flow (Figure 1).

Cyclohexanone at 1416 ppm caused a significant increase in cortical blood flow, beginning ~30 s after stimulus onset and continuing to remain significantly higher than clean air trials until ~20 s after the stimulus was turned off (Figure 1). No other concentration of cyclohexanone elicited a significant increase in cortical blood flow above controls.

Amyl acetate at 3020 ppm resulted in an average blood flow increase significantly higher than air, starting ~30 s after stimulus onset and continuing until ~30 s after the stimulus was terminated (Figure 1). In addition, amyl acetate at 955 ppm caused a significant increase in blood flow above clean air trials ~60, 100 and 110 s after the stimulus was turned on (Figure 1). The lowest concentration of amyl acetate (302 ppm) caused no significant increase in the blood flow above controls. No concentration of butanol presented to the animals resulted in a significant increase in blood flow above air trials.

The average heart rate of the 10 animals remained constant throughout the experiment, regardless of the odorant presented. In addition, the respiration rate remained constant throughout all experiments for all the animals.

Blood flow was recorded simultaneously from the frontal

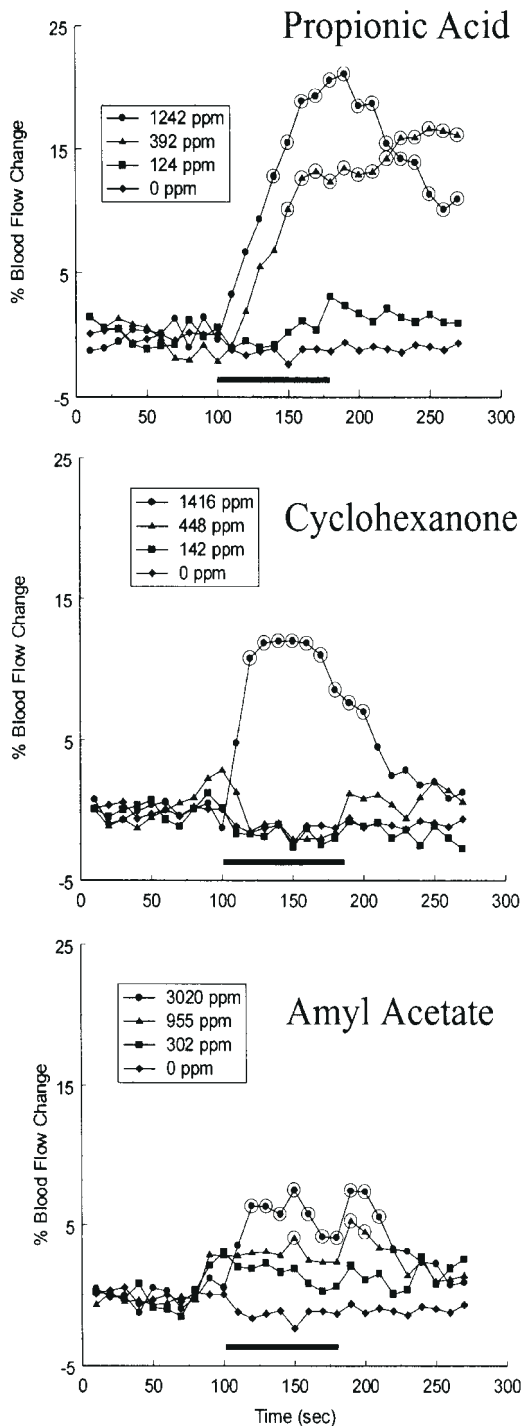


Figure 1 Mean percentage cortical blood flow change in response to the presentation of three odorants. Circled data points indicate a significant difference from the air trial (0 ppm) at the same time interval. The black bar beginning at 100 s indicates the stimulus presentation ($n = 10$).

and occipital cortices through two different burr holes in four of the animals tested. Odorant-elicited blood flow changes in the frontal cortex were highly correlated with changes in the occipital cortex ($r = 0.562$, $P < 0.001$).

Discussion

Cerebral blood flow is mainly dependent on vascular resistance when autoregulation is intact. Vessel tone is regulated by parenchymal factors (such as metabolites), endothelial factors and perivascular nerves (Wahl and Schilling, 1993). In the present study, the odorant-induced blood flow changes recorded in the frontal and occipital cortices were highly correlated. This finding suggests that blood flow increases were not mediated by local parenchymal or endothelial factors but rather by perivascular nerves.

A systemic change in vessel caliber could be induced by the odorants entering the blood stream. If this were the case, there would be a corresponding decrease in systemic blood pressure which could affect blood flow. Since blood pressure was not measured in this study, this possibility cannot be ruled out. However, the time-course for cortical blood flow increase was very similar to that seen during electrical stimulation of fibers innervating cerebral blood vessels (Suzuki *et al.*, 1990a, 1990b), suggesting perivascular nerve stimulation.

Nerves responsible for regulating cerebral vessel caliber include sympathetic fibers, parasympathetic fibers and sensory fibers of the trigeminovascular system (Wahl and Schilling, 1993). Stimulation of sympathetic fibers results in minor to modest decreases in cerebral blood flow (Baumbach and Heistad, 1983), while stimulation of parasympathetic fibers or trigeminal fibers results in increases in cerebral blood flow (Suzuki *et al.*, 1990a,b). The significant increase in blood flow caused by three of the four odorants presented suggests that parasympathetic and/or trigeminal fibers were stimulated resulting in cerebral vessel dilation.

Propionic acid, cyclohexanone, amyl acetate and butanol are known to stimulate both olfactory and trigeminal nerves at the concentrations used in the present study (Silver, 1992). Since olfactory stimuli can elicit changes in autonomic function (Cassel and Roberts, 1991), the blood flow increases elicited by these odorants could have been caused by parasympathetic stimulation via the olfactory nerve. However, odorant stimulation of the trigeminal nerve may be more likely to lead to increases in cerebral blood flow. Trigeminal ganglion stimulation in the cat results in increased cerebral blood flow (Lambert *et al.*, 1984). Electrical stimulation of the nasociliary nerve in rat also increases cerebral flow (Suzuki *et al.*, 1990a). In addition, significant increases in blood flow required relatively high concentrations of odorants. Typically, the odorants used in this study stimulate olfactory receptors at much lower concentrations and trigeminal nerve stimulation requires higher concentrations (Silver, 1992).

It is not clear why butanol did not elicit changes in cerebral blood flow in the present study, since the concentrations used should have been high enough to stimulate trigeminal nerve fibers. However, previous work in our laboratory has shown that butanol, at the concentrations

tested, elicits the weakest ethmoid nerve response compared with the concentrations of the other three odorants used in this study.

Blood flow increases due to trigeminal nerve stimulation may be mediated by parasympathetic fibers. The parasympathetic nerves that innervate cerebral blood vessels arise from the sphenopalatine ganglion, which is known to be innervated by trigeminal fibers (Suzuki *et al.*, 1989). The trigeminal nerve may also mediate cerebral blood flow changes directly. Trigeminal fibers that innervate cerebral vessels may be collaterals from the same fibers that innervate the nasal cavity, since trigeminal fibers innervating the nasal cavity have been shown to project collaterals back into the cranium (Finger and Böttger, 1993). Additional evidence to support a connection between nasal trigeminal and cerebrovascular trigeminal fibers involves capsaicin application to the nasal mucosa as a treatment for cluster headache. Cluster headaches are severe unilateral attacks of head pain that occur in a series of episodes. Symptoms include blockage of nasal passages and rhinorrhea. Studies by Fusco *et al.* (Fusco *et al.*, 1994) have shown that capsaicin applied to the nasal mucosa of humans causes changes in cerebral blood flow in addition to significantly decreasing the number of headaches in patients suffering from cluster headache. These findings tend to suggest that the desensitizing actions of capsaicin may reduce the frequency of cluster headache because collaterals of the same trigeminal nerve may innervate both the nasal cavity and cerebral blood vessels.

The results of the present study indicate that odorants presented to the nasal cavity can elicit significant increases in cortical blood flow. Clearly, the underlying pathological mechanisms for headache are complex and not completely understood; however, since odorants can trigger vascular headaches (Blau and Solomon, 1985; Hudnell *et al.*, 1992) and stimulation of the trigeminovascular system results in both head pain and increased cortical blood flow (Ray and Wolff, 1940; Lambert *et al.*, 1984; Suzuki *et al.*, 1990a), the headache elicited by odorants may be due to activating the trigeminovascular system. Further experiments are needed to determine whether this activation is via the olfactory or trigeminal systems.

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References

- Baumbach, G. and Heistad, D.D. (1983) *Effects of sympathetic stimulation and changes in arterial pressure on segmental resistance of cerebral vessels in rabbits and cats*. *Circ. Res.*, 52, 527–533.
- Blau, J.N. and Solomon, F. (1985) *Smell and other sensory disturbances in migraine*. *J. Neurol.*, 232, 275–276.
- Cassell, M.D. and Roberts, L. (1991) *Ultrastructural evidence for an olfactory-autonomic pathway through the rat central amygdaloid nucleus*. *Neurosci. Lett.*, 133, 100–104.
- Finger, T.E. and Böttger, B. (1993) *Peripheral peptidergic fibers of the trigeminal nerve in the olfactory bulb of the rat*. *J. Comp. Neurol.*, 334, 117–124.
- Fusco, B.M., Fiore, G., Gallo, F., Martelletti, P. and Giacobuzzo, M. (1994) *"Capsaicin-sensitive" sensory neurons in cluster headache: pathophysiological aspects and therapeutic indication*. *Headache*, 34, 132–137.
- Hudnell, H.K., Otto, D.A., House, D.E. and Mølhave, L. (1992) *Exposure of humans to a volatile organic mixture. II. Sensory*. *Arch. Environ. Health*, 47, 31–38.
- Lambert, G.A., Bogduk, N., Goadsby, P.J., Duckworth, J.W. and Lance, J.W. (1984) *Decreased carotid arterial resistance in cats in response to trigeminal stimulation*. *J. Neurosurg.*, 61, 307–315.
- Maggi, C.A. and Meli, A. (1988) *The sensory-efferent function of capsaicin-sensitive neurons*. *Gen. Pharmacol.*, 19, 1–43.
- Moskowitz, M.A. (1991) *The visceral organ brain: implications for the pathophysiology of vascular head pain*. *Neurology*, 41, 182–186.
- Morita, Y., Hardebo, J.E. and Bouskela, E. (1995) *Influence of cerebrovascular sympathetic, parasympathetic, and sensory nerves on autoregulation and spontaneous vasomotion*. *Acta Physiol. Scand.*, 154, 121–130.
- Nakashima, T., Asami, K., Akanabe, K., Kuno, K., Nagai, H. and Yanagita, N. (1995) *Effect of carbon dioxide on facial nerve blood flow in rabbits*. *Acta Otolaryngol. Stockh.*, 115, 40–43.
- Ray, B.S. and Wolff, H.G. (1940) *Experimental studies on headache: pain sensitive structures of the head and their significance in headache*. *Arch. Surg.*, 41, 813–856.
- Saito, K., Liu-Chen, L.-Y. and Moskowitz, M.A. (1987) *Substance P-like immunoreactivity in rat forebrain leptomeninges and cerebral vessels originates from the trigeminal but not sympathetic ganglia*. *Brain Res.*, 403, 66–71.
- Silberstein, S.D. (1992) *Advances in understanding the pathophysiology of headache*. *Neurology*, 42 (Suppl. 2), 6–10.
- Silver, W.L. (1992) *Neural and pharmacological basis for nasal irritation*. In Tucker, W.G., Leaderer, B.P., Mølhave, L. and Cain, W.S. (eds), *Sources of Indoor Air Contaminants*. *Ann. NY Acad. Sci.*, 641, 152–163.
- Silver, W.L., Walker, D.B., Ogden, M.W. and Walker, J.C. (1990) *Nasal trigeminal responses to toluene presented by an automated delivery system*. *Chem. Senses*, 15, 701–712.
- Skarphedinsson, J.O., Harding, H. and Thorén, P. (1988) *Repeated measurements of cerebral blood flow in rats. Comparisons between the hydrogen clearance method and laser Doppler flowmetry*. *Acta Physiol. Scand.*, 134, 133–142.
- Suzuki, N., Hardebo, J.E. and Owman, Ch. (1989) *Trigeminal fibre collaterals storing substance P and calcitonin gene-related peptide associate with ganglion cells containing choline acetyltransferase and vasoactive intestinal polypeptide in the sphenopalatine ganglion of the rat. An axon reflex modulating parasympathetic ganglionic activity?* *Neuroscience*, 30, 595–604.
- Suzuki, N., Hardebo, J.E., Kåhrström, J. and Owman, Ch. (1990a)

Effect on cortical blood flow of electrical stimulation of trigeminal cerebrovascular nerve fibres in the rat. Acta Physiol. Scand., 138, 307–315.

Suzuki, N., Hardebo, J.E., Kåhrström, J. and Owman, Ch. (1990b) *Selective electrical stimulation of postganglionic cerebrovascular parasympathetic nerve fibers originating from the sphenopalatine ganglion enhances cortical blood flow in the rat.* J. Cereb. Blood Flow Metab., 10, 383–391.

Tsai, S.-H., Tew, J.M., McLean, J.H. and Shipley, M.T. (1988) *Cerebral arterial innervation by nerve fibers containing calcitonin gene-related peptide (CGRP): I. Distribution and origin of CGRP perivascular innervation in the rat.* J. Comp. Neurol., 271, 435–444.

Wahl, M. and Schilling, L. (1993) *Regulation of cerebral blood flow—a brief review.* Acta Neurochir. 5 (Suppl.), 3–10.

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