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The effect of CO₂- and O₂-gas mixtures on laser Doppler measured cochlear and skin blood flow in guinea pigs

Johanna Kallinen¹, Anne Didier², Josef M. Miller², Alfred Nuttall² and Reidar Grénman¹

¹ University Central Hospital Department of Otolaryngology, Turku, Finland and ² Kresge Hearing Research Institute The University of Michigan, Ann Arbor, Michigan, U.S.A.

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The effects of carbogen (5% CO₂:95% O₂), 10% CO₂-in-air and 100% O₂ on cochlear blood flow (CBF), skin blood flow (SBF), blood pressure (BP) and arterial blood gases were investigated in the anesthetized, respired or self-respiring guinea pig. In respired animals, CBF and SBF were increased with carbogen and 10% CO₂-in-air and decreased with O₂. BP was elevated with each gas. In freely breathing animals, only 10% CO₂-in-air caused a small increase in CBF; both carbogen and O₂ caused CBF to decrease. SPF changes were similar in form, but larger than those seen in respired subjects. No consistent change in BP was seen during breathing of these mixtures.

Arterial PO₂ was increased by carbogen and 10% CO₂-in-air for both groups. PCO₂ increased for both CO₂ gas mixtures during forced respiration; but in free-breathing animals PCO₂ only increased for 10% CO₂-in-air (normal PCO₂ values were maintained with carbogen through increased breathing rate). The observed changes in CBF were consistent with a balance between a combined vasoconstrictive effect of PO₂ and vasodilation effect of PCO₂ on cochlear vessels. Analysis of cochlear vascular conductivity (CBF/BP) indicated that vasodilation was significant only with 10% CO₂-in-air in respired animals. In all other conditions the increased CBF apparently reflects the increase perfusion pressure associated with respiration of each gas. For clinical purposes, while carbogen does not appear to directly cause vasodilation of cochlear vessels it does lead to an increased oxygenation of the cochlea blood and would appear to avoid the cochlear vasoconstriction caused by 100% O₂.

CO₂; O₂; Cochlea blood flow; Skin blood flow; Blood gases; Guinea pig

Introduction

Carbon dioxide is known to have a powerful vasodilative effect on the smooth muscle of blood vessels (Duling, 1973; Jackson et al., 1974; Olesen, 1974; Sadoshima et al., 1980). It also activates the sympathetic nervous system causing epinephrine and norepinephrine to be released (Elam et al., 1981). However, the adrenergic vasoconstrictive effect is smaller than the direct effect of carbon dioxide on the smooth muscles resulting in an overall vasodilation. Diji (1959) demonstrated vasodilation by carbon dioxide of blood vessels in the extremity by measuring the heat elimination from the hand to water at 29°C when the water was saturated with carbon dioxide. He attributed the observed rise of water temperature to local vasodilation. Radawski et al. (1972) demonstrated a decrease in small vessel resistance in the skin of the forelimb of

dogs as arterial PCO₂ increased. The effect of CO₂ on cutaneous microcirculation has also been measured by laser-Doppler flowmetry (LDF) (Schnizer et al., 1985) and the authors concluded that blood flow (BF) depends on CO₂-concentration and on skin temperature. However, Svensson et al. (1983), using a LDF to study skin blood flow (SBF) in fingers, could not demonstrate a direct correlation between BF and transcutaneous PCO₂ values within a physiological range.

In animal studies of brain circulation, by 1930 the effects of CO₂ were well documented (Wolff and Lennox, 1930; see also Harper and Glass, 1965). In humans, the influence of hyper- or hypocapnia on cerebral flow has also been demonstrated (Patterson et al., 1955; Severinghaus and Lassen, 1967). More recently, Hansen et al. (1984) found a significant increase in total brain BF during hypercapnia in their study of the effects of variations in PCO₂ in newborn piglets; and Rosenberg (1988) showed cerebral vasoconstriction with hypocapnia in newborn lambs using radioactive microspheres.

The effect of CO₂ on the inner ear has long been of interest, because of the potential of this agent to increase BF in otological diseases and trauma where a vascular dysfunction is thought to be a contributing factor, i.e. Ménière's disease, acoustic trauma and,

Correspondence to: Josef M. Miller, Kresge Hearing Research Institute, The University of Michigan, 1301 East Ann Street, Ann Arbor, MI 48109-0506, U.S.A. Fax: (313)-764-0014.

Abbreviations: LDF laser-Doppler flowmetry; BF blood flow; SBF skin blood flow; CBF cochlear blood flow; L-D laser-Doppler; ABF average baseline flow; BP blood pressure.

most importantly, some forms of sudden deafness. The presumption is that, increasing cochlear blood flow (CBF) will improve oxygenation of cochlear tissues and, hence, enhance their resistance to damage and their repair following the trauma. Carbon dioxide inhalation, as a treatment, has been used in sudden deafness and noise-induced hearing loss (Wilkins et al., 1987; Fisch, 1983; Brown et al., 1982; Giger, 1979). These authors suggest that this treatment is effective in the amelioration of hearing loss. However, the spontaneous recovery rates of these pathologies are high; and thus, it is difficult to interpret results from such studies reliably.

Using plethysmography, Morimitsu (1960) and Suga and Snow (1969) found an increase of CBF in guinea pigs with inhalation of 100% CO₂. Pollock et al. (1974) observed increased BF in the temporal bones of dogs with enriched CO₂ inhalation. Hultcrantz et al. (1980), using the microsphere technique, demonstrated that 7% CO₂-in-air is more effective in increasing CBF than 7% CO₂ + 93% O₂ or 10% CO₂-in-air. Dengerink et al. (1984) demonstrated that carbogen (5% CO₂ + 95% O₂) inhalation increases BF and causes a limited vasodilation in their study of effects of noise and carbogen on cochlear vasculature. Moreover, Fisch (1976, 1983) found an increased oxygenation of perilymph in cats and humans associated with breathing carbogen, and Prazma et al. (1979) demonstrated increased PO₂ in guinea pig's endolymph during forced respiration of 5–10% CO₂ in O₂. Of course, some of these effects may be explained by the breathing of an enriched O₂ mixture, but, the observations generally tend to support the basic rationale of enhanced CBF. Questions remain concerning the most effective CO₂ gas concentration, the influence of the respiration protocol (forced vs. free-breathing) and the physiological mechanisms involved.

Indeed, in at least one study, CO₂ breathing was found to have little effect on LDF measured CBF (Miller et al, 1991). In related work, LaRoucre et al. (1989) compared changes of LDF and intravital microscopy measured red cell velocity, finding that the changes depended upon the concentration of CO₂ and whether the gas was provided under conditions of free-respiration or forced ventilation.

The purpose of the current study was to investigate the effects of carbon dioxide and oxygen on inner ear BF in guinea pigs using LDF and to assess the influence of free- and forced-respiration on these effects.

Materials and Methods

Subjects

Fourteen young healthy pigmented guinea pigs of both sexes weighing 200–450 g were used in this study.

All procedures of the investigation were evaluated and approved by The University of Michigan's Committee on Use and Care of Animals; animals were housed and cared for by the University of Michigan's Unit for Lab Animal Medicine, which is accredited by the American Association for Laboratory Animal Science. The subjects were divided into two groups: group I (9 animals) were ventilated by a Harvard rodent ventilator (Model 683) and group II (5 animals) were freely breathing.

Surgical procedures and preparation

The animals were anesthetized with pentobarbital (Nembutal 0.6 ml/kg IP) and a fentanyl/Droperidol mixture (Innovar-Vet, 0.4 ml/kg IM) 10–15 min later. Additional doses of Nembutal and Innovar were administered every 2 h and every hour, respectively, throughout the experiment to maintain the appropriate level of anesthesia. Core body temperature was held constant with a heating pad regulated with a rectal probe sensor. A tracheotomy was performed and a cannula inserted in the right carotid artery to allow blood pressure (BP) measurements and blood sample withdrawals.

A ventral surgical approach was performed to expose the middle ear of the animals. The mucosa of the cochlea was gently removed with a cotton pledget, permitting placement of the measurement probe directly against the bony surface. CBF recordings were then obtained with a laser-Doppler (L-D) probe (TSI Corp Laserflo, Minnesota) placed on the bony wall of the basal turn of the cochlea. In addition, hair was shaved on a small area on the flank to allow placement of a second L-D probe (Perimed PF-1 AB, Sweden) to monitor SBF.

Experimental protocol

All the animals received 5% CO₂ + 95% O₂ (carbogen), 10% CO₂-in-air, and 100% O₂ each for 10 min. An interval of at least 10 min occurred between gas administrations. A stable CBF baseline measurement of at least 3 min was recorded at the beginning of each experiment and before inhalation of each gas mixture.

Group I was ventilated with a rodent ventilator (Harvard rodent ventilator model 683) with a stroke volume of approximately 2.6cc, at 42 strokes/min. Group II breathed the gases freely through a plastic tube, which loosely surrounded the tracheotomy tube.

Measures and analysis

For oxygen (PO₂) and carbon dioxide tension (PCO₂) measurements 0.2 ml of arterial blood was taken via carotid artery cannula 2–3 min before each gas inhalation and 5 min after gas inhalation began. For each animal, BP, CBF, SBF, and a calculation of the instantaneous ratio of CBF to BP (the normalized CBF which is an estimate of total cochlear vascular

conductivity) were recorded on a strip chart throughout the experiment. These records were quantified for a 3-min period preceding gas inhalation, a 10-min period of gas inhalation, and for 4 min after discontinuing gas inhalation, or until the values returned to baseline. Since the output of the LDF provides a proportional measure of flux, not yet quantifiable in physical units, the effect of respiration of different gas mixtures on BF was evaluated in terms of percent change in flow from the average baseline flow (ABF). For comparison, BP and normalized CBF has also been presented as percentile changes of the averaged baseline level. Note that for the purpose of analysis, all three measures (CBF, BP and normalized CBF) are taken as percentage of baseline from the chart recorder record at one-minute intervals. The values were then averaged across animals.

Results

Group I (respirated animals)

Fig. 1 illustrates the mean CBF changes for guinea pigs receiving carbogen, 10% CO₂-in-air, or 100% oxygen. Both carbogen and 10% CO₂-in-air induced an increase in CBF starting within the first minute of the inhalation period. The mean maximum increase, 15% for carbogen and 27% for CO₂-in-air, was reached within 2 min. After interruption of the carbogen breathing, CBF returned to baseline within 2 to 3 min. The recovery after 10% CO₂-in-air took up 6 to 7 min. In contrast with the effect of the CO₂ gas mixtures, oxygen induced approximately a 12% decrease in CBF that returned to baseline within 6 min.

SBF changes are shown on Fig. 2. They exhibited a similar pattern as the CBF changes; however, the la-

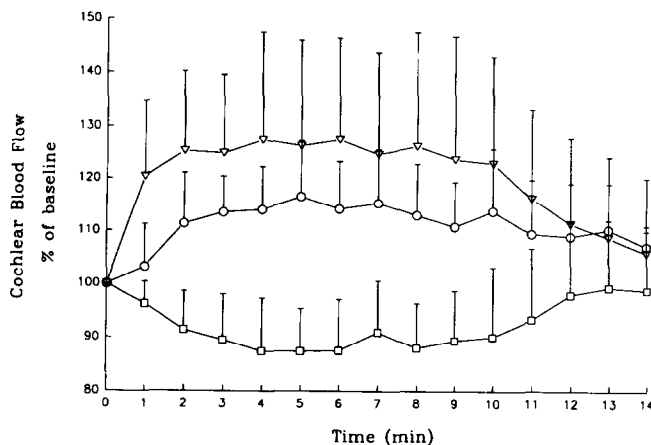


Fig. 1. Mean change in CBF during and following 10 min of respiration (forced respirated) of Carbogen (○-○) ($N = 5$), 100% O₂ (□-□) ($N = 4$), or 10% CO₂-in-air (▽-▽) ($N = 5$). Gas administration was started at 0 min time. Vertical bars are one standard deviation above and below the mean.

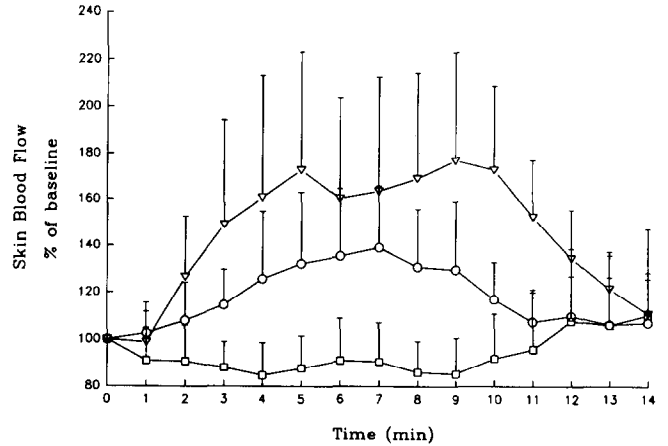


Fig. 2. Mean change in SBF. Same animals and conditions as in Fig. 1.

tency of response to maximum change was slower and the magnitude of the oxygen-induced decrease (18%), as well as the increases induced by carbogen (36%) and the 10% CO₂-in-air (77%), were larger than the CBF changes in the same conditions. The inhalation of all three gas mixtures increased the BP (Fig. 3). The maximum mean increase was 6% with oxygen, 19% with carbogen and 27% with 10% CO₂-in-air.

Group II (Freely breathing animals)

In contrast to group I, carbogen respiration in the free-breathing group induced a 12% decrease in CBF; and 10% CO₂-in-air induced only a 6% increase in CBF (Fig. 4). With oxygen, CBF decreased more than 20%. SBF changes were similar to those observed in the group I, although larger in magnitude (Fig. 5). Most dramatic were the changes observed with 10% CO₂-in-air: SBF increased more than 160% within 2 min. In the freely breathing animals, O₂ caused a small increase in SBF, as opposed to the small decrease observed in the respirated animals. BP did not vary

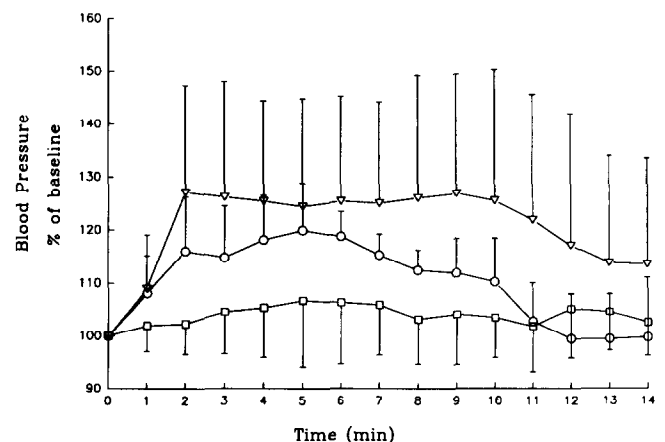


Fig. 3. Mean change in systemic BP. Same animals and conditions as in Fig. 1.

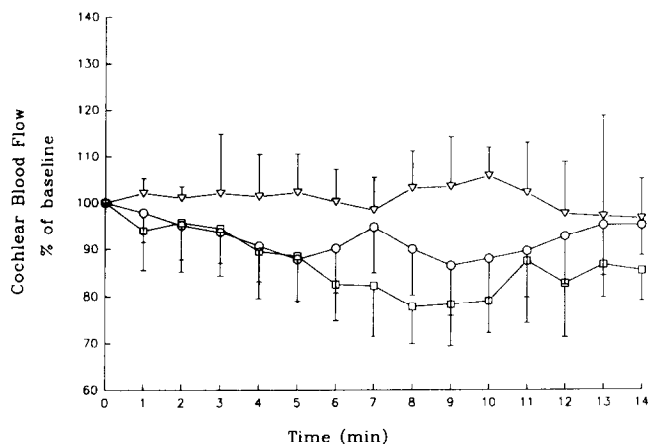


Fig. 4. Mean change in CBF during and following a 10 min period of free-breathing with Carbogen (○-○) (N = 6), 100% O₂ (□-□) (N = 8), or 10% CO₂-in-air (▽-▽) (N = 8). Gas administration was started at 0 min time. Vertical bars are one standard deviation above and below the mean.

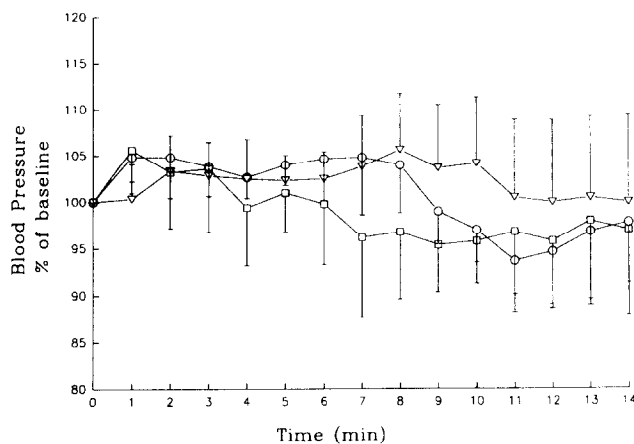


Fig. 6. Mean change in systemic BP during and following a 10 min period of free-breathing with Carbogen (○-○) (N = 9), 100% O₂ (□-□) (N = 8), or 10% CO₂-in-air (▽-▽) (N = 8). Gas administration was started at 0 min time. Vertical bars are one standard deviation above and below the mean.

systematically during respiration of the oxygen in the freely breathing animals (Fig. 6), it increased less than 10% with carbogen and the 10% CO₂-in-air in this subject group.

Blood gas values

Arterial blood gas values of PO₂ and PCO₂ for the individual animals are shown in Table I for group I and Table II for group II. The mean changes, as a percent of control levels, are given in Table III. Changes in PO₂ and PCO₂ values reflected the composition of the administered gas mixture and depended on the experimental condition of respiration. Thus in group I, PO₂ values are greatly elevated during respiration of enriched oxygen mixtures while PCO₂ values are elevated by respiration of carbogen and the 10% CO₂-in-air mixture. In group I, the control PCO₂ values are slightly

reduced, by comparison to baseline values observed in group II, by the forced ventilation rate (i.e., a small hyperventilation), which allowed the distinct PCO₂ elevation to occur during respiration of both gas mixtures containing CO₂. The animals of group II demonstrated elevated PO₂ values during respiration of the enriched oxygen measures, while PCO₂ values were elevated only during respiration of the 10% CO₂-in-air mixture. The animals respiring 10% CO₂-in-air not only demon-

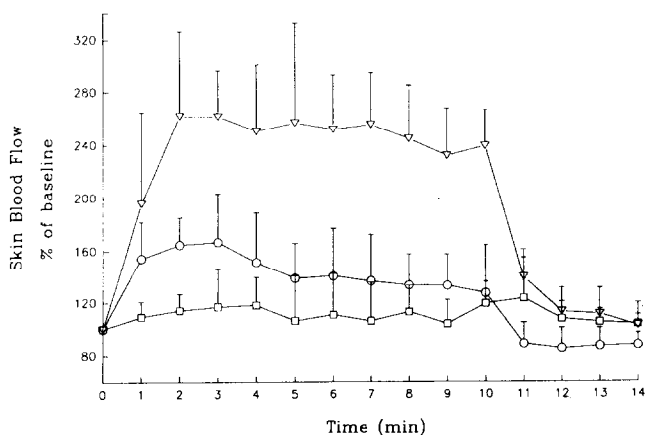


Fig. 5. Mean change in SBF during and following a 10 min period of free-breathing with Carbogen (○-○) (N = 6), 100% O₂ (□-□) (N = 6), or 10% CO₂-in-air (▽-▽) (N = 8). Gas administration was started at 0 min time. Vertical bars are one standard deviation above and below the mean.

TABLE I
ARTERIAL BLOOD GAS VALUES FOR ANIMALS IN GROUP ONE

	PO ₂ Oxygen (mmHg)		PCO ₂ Carbon Dioxide (mmHg)	
	Baseline	Respired	Baseline	Respired
Carbogen	111.8	315.9	29.3	42.5
	94.4	323.6	30.3	50.1
	54.2	256.0	34.0	50.3
	74.2	255.0	31.2	45.0
	76.3	290.1	44.8	62.9
	122.8	311.3	26.8	37.3
10% CO ₂ in-air	101.1	232.5	23.7	37.8
	101.9	121.7	26.0	52.0
	94.8	99.5	24.7	65.0
	75.2	73.7	27.2	45.7
	112.5	107.7	25.6	48.9
	94.9	94.4	33.0	53.0
100% O ₂	111.6	94.4	27.5	59.3
	116.8	94.7	19.9	48.2
	113.0	380.2		
	101.9	449.5	26.6	26.4
	76.9	325.0	25.5	22.2
	96.2	313.7	29.1	23.9
	97.5	398.0	38.5	29.5
	115.5	366.6	25.3	26.2
	107.5	334.4	22.4	21.0

TABLE II
ARTERIAL BLOOD GAS VALUES FOR ANIMALS IN GROUP TWO

	PO ₂ Oxygen (mmHg)		PCO ₂ Carbon Dioxide (mmHg)	
	Baseline	Respired	Baseline	Respired
Carbogen	95.2	393.4	51.0	50.7
	79.9	373.7	50.4	58.3
	88.6	349.1	39.5	44.4
	86.4	199.4	44.1	40.6
	89.7	230.2	45.9	44.8
10% CO ₂ in-air	103.9	152.2	54.7	72.7
	72.0	114.9	52.7	91.8
	98.3	145.1	39.9	57.7
	54.0	64.0	26.4	39.4
	92.3	159.9	40.6	44.5
100% O ₂	112.2	405.6	43.3	40.9
	130.4	432.9	31.0	35.2
	82.0	277.8	35.0	38.0

TABLE III
MEAN CHANGES IN ARTERIAL BLOOD GAS VALUES AS A PERCENT OF CONTROL LEVELS

	PO ₂ (% of baseline)		PCO ₂ (% of baseline)	
	Respirated	Non-Respirated	Respirated	Non-Respirated
Carbogen	352 ± 81	352 ± 103	148 ± 11	103 ± 10
10% CO ₂ in-air	98 ± 13	149 ± 20	206 ± 37	142 ± 24
Oxygen	366 ± 55	344 ± 15	90 ± 10	105 ± 10

strated high arterial PCO₂ levels, but elevated PO₂ values as well, due to hyperventilation. It is clear from Table III that the greatest increase in PO₂ occurred for the guinea pig freely respiring 100% oxygen and the greatest PCO₂ increases were obtained by respiration of 10% CO₂-in-air.

Discussion

Carbon dioxide has been used in the treatment of some forms of hearing loss e.g., sudden hearing loss. The physiological rationale is based on the presumed vasodilation of cochlear vessels leading to enhanced CBF and oxygenation of inner ear tissues or facilitation of the removal of waste products in the traumatized ear.

In the current study, we have demonstrated that in respirated guinea pigs (Group I) L-D measured CBF is increased by inhalation of CO₂ (Fig. 1). Moreover, it was observed that 10% CO₂-in-air was more effective than Carbogen. These data are consistent with the

finding of Hultcrantz et al. (1980) that 7% CO₂-in-air was more effective than carbogen in increasing microsphere measured CBF. It is noteworthy that an increase in BF is seen with carbogen (containing 95% O₂) respiration in spite of the finding that respiration with 100% oxygen resulted in a decrease in CBF. Thus, the difference in response between the two carbon dioxide containing gases may be attributed to the vasodilation effect of the 5% CO₂, whereas oxygen has been shown to be a vasoconstrictor (Ishikawa et al. 1980; Lombard and Stekiel, 1988; Sybertz et al. 1986).

The free-breathing animals (Group II) had smaller and more variable responses (Fig. 4). The most consistent and strongest CBF change in these animals was the decrease seen with 100% O₂ breathing which again supports the notion that O₂ has an important vasoactive role in the ear.

The percentage changes given in Table III for PO₂ and PCO₂ provide a basis for explaining the CBF changes observed with these gases. Arterial PO₂ is substantially increased during Carbogen or 100% O₂ respiration regardless of whether the animal is freely breathing or ventilated. PCO₂ values were increased for both CO₂ gas mixtures during forced respiration; but in the free-breathing condition, PCO₂ only increased when the concentration was 10%-in-air. The concentration of PCO₂ in the blood presumably reflects the ability of the animal to control the CO₂ under free-breathing conditions. If PCO₂ causes vasodilation in the ear and PO₂ causes constriction, we may predict that CBF changes with these gases will be a function of the combined arterial levels of each. Thus, the greatest increase in CBF is expected with forced respiration of 10% CO₂-in-air (largest change in PCO₂ and no change in PO₂) and the largest decrease in CBF, with forced respiration of 100% O₂. The resultant changes in CBF are consistent with the vascular levels of these gases and supported by the findings of LaRouere et al., (1989).

In order to further assess the mechanism underlying CO₂ induced CBF changes, one would like to examine directly the resistance of the vessels of the cochlea. Vascular resistance can be approximated by a correction to remove the flow alterations due to BP change alone (Sillman et al., 1988). It is clear from Figs. 3 and 6 that substantial BP changes occurred in all cases. We normalized the L-D measured CBF values by electronically dividing (in real time) the output signal of the LD (CBF) by the output of the BP transducer for each subject. To the extent that the systemic BP measure reflects perfusion pressure at the cochlea, this value (flow/pressure) is equivalent to whole organ vascular conductivity and this is plotted in Fig. 7. Fig. 7a, which indicates normalized changes under forced respiration conditions, demonstrates that net vasodilation of the cochlea vessels only appears significant during forced

respiration with the 10% CO₂-in-air mixture (during which PCO₂ increased 100% over baseline levels). Thus, the increased CBF changes observed with carbogen reflect the increased perfusion pressure associated with forced respiration of this gas; just as the increased CBF observed with 10% CO₂-in-air reflects an increased perfusion pressure plus vasodilation. The vasoconstriction expected from respiration with pure oxygen is also evident in Fig. 7a. Under conditions of free-breathing (Fig. 7b), the decreased flow observed with 100% O₂ and carbogen gas both primarily reflect a vasoconstriction induced by the high concentrations

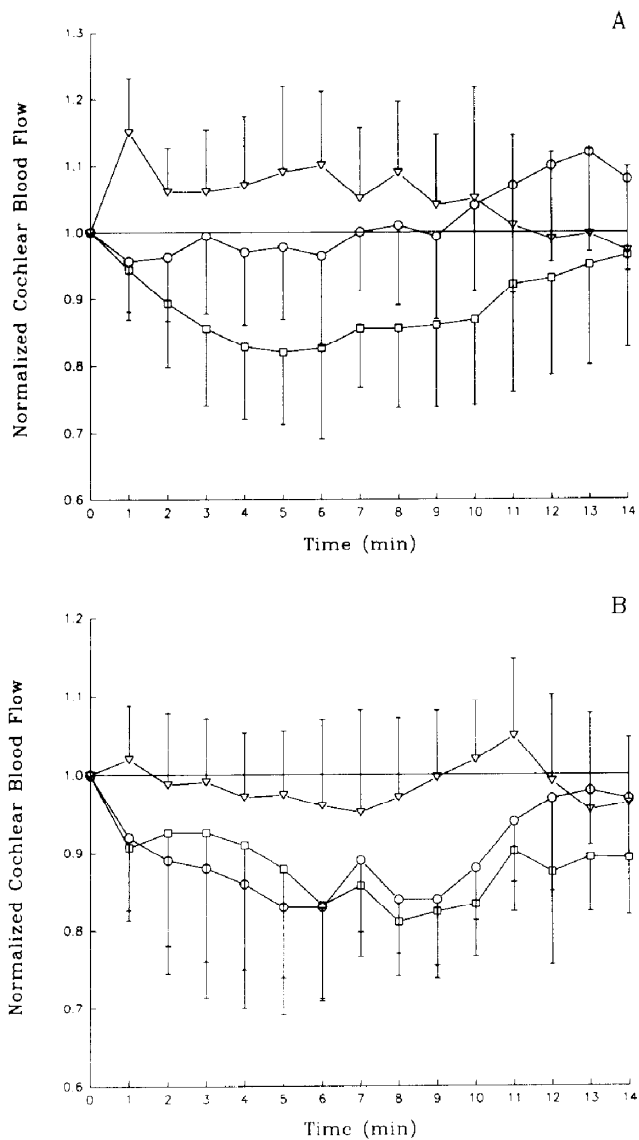


Fig. 7. Mean (\pm one standard deviation) normalized CBF (CBF/BP) during and following 10 min of respiration with various gas mixtures. A) Forced respiration: Carbogen (○-○) (*N* = 6), 100% O₂ (□-□) (*N* = 5), 10% CO₂-in-air (▽-▽) (*N* = 4). B) Free breathing: Carbogen (○-○) (*N* = 5), 100% O₂ (□-□) (*N* = 4), 10% CO₂-in-air (▽-▽) (*N* = 5).

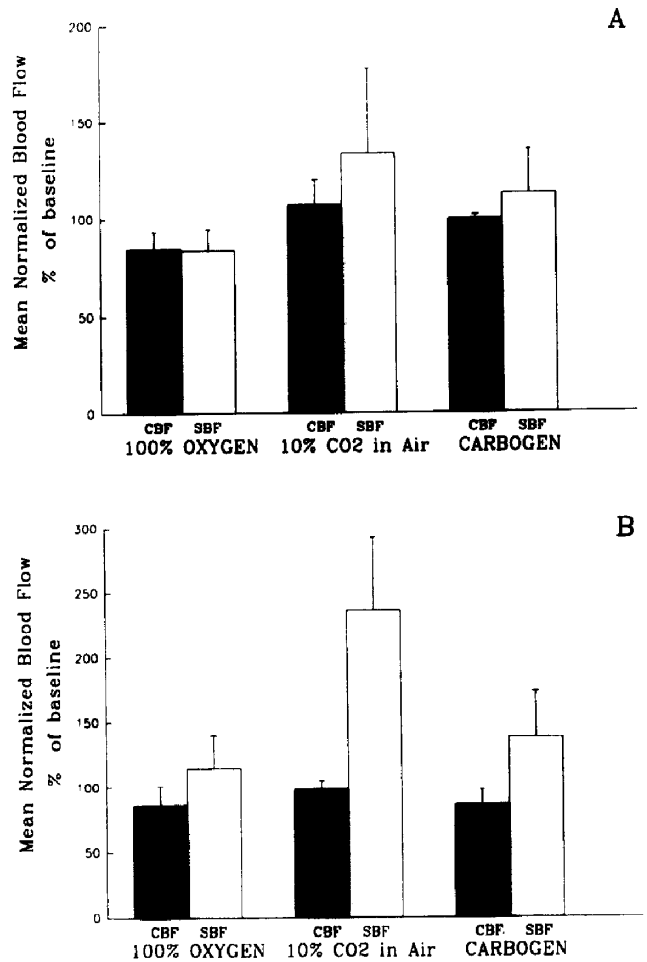


Fig. 8. Comparison of the mean change in normalized BF of the skin and the cochlea; a) forced respiration, b) free-breathing. Data were calculated from the last 7 min of the respiration time period. Bars are 95% confidence intervals.

of O₂ (associated with increase of approximately 150% in arterial PO₂).

The changes in vascular conductivity (normalized BF) seen in the skin with CO₂ respiration were consistently much larger than those observed for the cochlea (Fig. 8). This confirms the finding by many earlier studies, that CO₂ is a potent vasodilator for smooth muscle in the skin (e.g., Schnizer et al. 1985; Svensson et al., 1983) and suggests that cochlear vessels are not as strongly influenced by arterial PCO₂. However, such a generalized interpretation must be taken with some care since at least three factors may influence this hypothesis. There may be 1) significant physical and physiological differences between skin and cochlear vasculature that would contribute to the LD flow measurement differences (e.g., there may be differences in vascular reactivity to circulating hormones affected by gas respiration or the local effects of vaso-regulators such as pH and adenosine), 2) BP at the labyrinthine artery may be significantly different from systemic BP and 3) the two L-D instruments may perform differ-

ently. Of these three factors, we view the latter as less likely since various studies have shown that LDFs are linear in their response to changes in BF (Nilsson et al., 1980; Baldwin et al., 1991).

Clinically, the current results suggest that administration of carbogen to patients by free-breathing does little to directly increase CBF, but the intervention may be clinically effective because of the delivery of an enriched O₂ blood to the cochlear tissues. Since under free-breathing conditions it appears that 5% CO₂ is inadequate to sufficiently increase PCO₂ to cause vasodilation, it could be argued to use only 100% O₂. However, this will lead to vasoconstriction of cochlear vessels. Clinically, this may be as unacceptable as is the use of 10% CO₂-in-air, which does result in an increase in vessel compliance, but which cannot be comfortably respired by awake patients. Indeed, if the treatment goal is to cause vasodilation of the cochlear vasculature, this approach would appear relatively ineffective. However, it may be just as important to increase tissue oxygenation for which the enriched O₂ mixtures are effective. Perhaps the advantage of 5% CO₂ in the enriched O₂ mixture is to partially offset the vasoconstrictive effect of the O₂ (Fig. 1, but not supported by data in Fig. 4). These considerations coupled with the Hultcrantz et al (1980) observations that 7% CO₂ produced the greatest CBF increase may indicate that the most useful strategy is to use O₂ with the maximum concentration of CO₂ that is safe and well tolerated.

One recent animal investigation of the effects of enriched oxygen breathing on the amelioration of a noise-induced hearing loss lends credence to this strategy. Hatch et al. (1990) found that respiration, during noise exposure, of either carbogen or 100% O₂ resulted in a dramatic reduction in the extent of temporary threshold shift produced by the noise exposure and the elimination, almost entirely, of a 40dB permanent threshold shift observed in control animals (breathing room air during noise exposure). Cochlear oxygenation will reflect two factors, total BF to the organ and O₂ exchange between tissues and red blood cells. Previous studies have indicated that fluid oxygen tension (perilymph and endolymph) of the cochlea is greatly increased during carbogen administration (e.g., Prazma, 1982). The current work indicates that this occurs without significant change in CBF and only minor change of BP. Therefore, carbogen may be the rational clinical choice for some patients.

Acknowledgements

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