Carotid body function in aged rats: responses to hypoxia, ischemia, dopamine, and adenosine

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Abstract The carotid body (CB) is the main arterial chemoreceptor with a low threshold to hypoxia. CB activity is augmented by A₂-adenosine receptors stimulation and attenuated by D₂-dopamine receptors. The effect of aging on ventilatory responses mediated by the CB to hypoxia, ischemia, and to adenosine and dopamine administration is almost unknown. This study

aims to investigate the ventilatory response to ischemia and to adenosine, dopamine, and their antagonists in old rats, as well as the effect of hypoxia on adenosine 3',5'-cyclic monophosphate (cAMP) accumulation in the aged CB. In vivo experiments were performed on young and aged rats anesthetized with pentobarbitone and breathing spontaneously. CB ischemia was induced

Scientific knowledge on the subject The functional ventilatory consequences of the morphological and molecular changes in the carotid body observed in aged animals, as well as the ventilatory effects of dopamine and adenosine in old animals, are still unknown.

What this study adds to the field The carotid body's peripheral control of ventilation is not impaired by aging. The preservation of the inhibitory and excitatory effects on ventilation caused by exogenous dopamine and adenosine, respectively, should be taken into account in the therapeutic use of these amines and their agonists and antagonists in elderly people.

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by bilateral common carotid occlusions. cAMP content was measured in CB incubated with different oxygen concentrations. Hyperoxia caused a decrease in cAMP in the CB at all ages, but no differences were found between normoxia and hypoxia or between young and old animals. The endogenous dopaminergic inhibitory tonus is slightly reduced. However, both the ventilation decrease caused by exogenous dopamine and the increase mediated by A_{2A}-adenosine receptors are not impaired in aged animals. The bradycardia induced by adenosine is attenuated in old rats. The CB's peripheral control of ventilation is preserved during aging. Concerns have also arisen regarding the clinical usage of adenosine to revert supraventricular tachycardia and the use of dopamine in critical care situations involving elderly people.

Keywords Aging · Peripheral chemoreceptors · Ventilation · Domperidone · cAMP · Adenosine A_{2A} receptors

Introduction

The carotid body (CB) is the main peripheral chemoreceptor organ that control ventilation sensing O₂ (PO₂), CO₂ (PCO₂), and pH in arterial blood, and initiate compensatory reflex responses via increased carotid sinus nerve (CSN) afferent activity, resulting in corrective changes to ventilation. CB glomus cells synaptically connected among them and with the afferent nerve terminals, depolarize in response to PO₂, PCO₂, and pH, releasing excitatory (acetylcholine, adenosine, and ATP) or inhibitory (dopamine) transmitters (Iturriaga and Alcaayaga 2004).

It is known that aging causes marked changes in the morphology of the CBs (Hurst et al. 1985) and reduces catecholamine release and CSN output in response to hypoxia in rat isolated CBs (Conde et al. 2006). In contrast, it has been suggested that peripheral chemoreceptor activity in healthy humans is not impaired by aging (Smith et al. 2001; Pokorski et al. 2004). Functional experiments in *in vivo* models to deal with the gap between *in vitro* isolated preparations of the CBs and humans are required.

Occlusions of the common carotid artery during short periods (s) have been used as a functional model for accessing peripheral chemoreceptor responses (Alcaayaga et al. 1986). The effects of aging on the

cardiorespiratory reflexes induced by carotid ischemia are yet to be studied.

Dopamine has inhibitory effects on ventilation during rest (Zapata and Zuazo 1980) and in the presence of hypoxic exposure (Nishino and Lahiri 1981), which are mediated by D₂-receptors located in the CB (Gonzalez et al. 1994). No data are available concerning the effects of aging on D₂-receptors at the CB.

A₂-adenosine receptors in the CB are involved in the stimulation of breathing in basal conditions (McQueen and Ribeiro 1986; Monteiro and Ribeiro 1987) and in response to hypoxia (Conde and Monteiro 2004).

In the present work, CB function was assessed by measuring the ventilatory response induced by acute CB ischemia and exogenous administrations of adenosine, and dopamine, and their receptor antagonists. Changes in cAMP content, in response to oxygen concentrations in aged rats, were also investigated as a cellular indicator of CB function. It has been postulated that cAMP modulates the response of the CB to hypoxia, and is the common activation pathway of both dopamine and adenosine receptors in the CB (Batuca et al. 2003). The effects of aging on CB cAMP production have never been addressed. An additional reason for testing this hypothesis is the age-related alterations in the adenylate cyclase/cAMP system, which has been documented in other structures. For example, impairment of the activity of the catalytic subunit of adenylate cyclase was observed in rats (Kilts et al. 2002), and in the heart of >60-year-old humans (Brodde et al. 1995). However, on the other hand, it has been described that the maximal ability of forskolin to increase cAMP in the adrenal medulla and liver is enhanced in 24-month-old rats (Tumer et al. 1996).

The results obtained in the present work will also contribute to investigate whether changes in the magnitude of the cardiorespiratory responses induced by dopamine and adenosine administration for clinical purposes (shock and supraventricular tachycardia, respectively) are to be expected in elder people.

Methods

Animals and house conditions

Experiments were performed on male Wistar rats: three groups aged 3 (*n*=36), 12 (*n*=18), and 24 (*n*=36) months completed the *in vivo* study; for the *in vitro*

study 30 carotid bodies were dissected from rats 3 ($n=7$), 12 ($n=7$), 18 ($n=4$), and 24 ($n=4$) months old. The animals were housed in the University's vivarium, in an air-conditioned room at $21\pm1^\circ\text{C}$, $55\pm10\%$ humidity, with a 12:12 h light/dark cycle (with lights on at 08:00 and off at 20:00), and with food and water available *ad libitum*. All surgical procedures and experimental protocols were handled in accordance with the EU guidelines for use of experimental animals (86/609/EEC). Efforts were made to minimize the number of animals used and their suffering. The investigators own FELASA level C certification and euthanasia of the rats was achieved by i.v. injection of a lethal dose of pentobarbital sodium (180 mg kg^{-1} iv).

In vivo experiments

A detailed description of these methods was previously published (Monteiro and Ribeiro 1987; Monteiro and Ribeiro 1989) and can be found in the online supplement. In short, respiratory frequency (f_R) and tidal volume (V_T) were obtained by pneumotachography. These respiratory parameters, blood pressure (BP), and heart rate (HR) were continuously recorded in anesthetized and vagotomized rats breathing spontaneously and submitted to either bilateral occlusions (5–15 s) of the common carotid artery or to drugs administered into the common carotid artery or femoral vein.

Bilateral midcervical vagotomy was performed in order to abolish the role of vagal afferents innervating the lungs and the aortic chemoreceptors with a major influence on respiratory activity (Marek et al. 2008) and can be influenced by drugs like adenosine (McQueen et al. 1998). Control experiments were performed in animals after bilateral denervation of the CBs by cutting the CSN to distinguish central and peripheral mediated effects.

Experimental protocols with cAMP

Immediately after surgical removal from the carotid bifurcation of anesthetized rats, the CBs were submitted to similar experimental conditions published elsewhere (Batuca et al. 2003) and described in the online supplement. Essentially, CBs were incubated in a solution, containing $500\text{ }\mu\text{M}$ of isobutylmethylxanthine equilibrated with $95\%\text{O}_2/5\%\text{CO}_2$ (hyperoxia), $20\%\text{O}_2/5\%\text{CO}_2/75\%\text{N}_2$ (normoxia), $10\%\text{O}_2/5\%\text{CO}_2/85\%\text{N}_2$ (mild hypoxia), or $5\%\text{O}_2/5\%\text{CO}_2/90\%\text{N}_2$

(moderately intense hypoxia), during 30 min. cAMP was assayed using an EIA commercial kit (GE Healthcare Bio-Sciences AB, Sweden).

Statistics

In *in vivo* experiments, each animal served as its own control. To simplify comparisons between young and old animals, cardiorespiratory data are expressed as percent changes. Data are expressed as $\text{mean}\pm\text{SEM}$ and statistical significance was evaluated by using the Student's paired and unpaired *t* test for *in vivo* experiments and one-way analysis of variance for *in vitro* experiments, with *p* values <0.05 taken as significant. The models for analysis were developed using GraphPad Prism software (Version 4.03).

Drugs

All drugs were prepared on the day of each experiment. The dosage of all drugs was calculated based on salt weight. Dopamine, adenosine, and domperidone were prepared in saline (0.9% NaCl). Stock solutions (5 mM) of SCH 58261 was prepared in dimethylsulfoxide and stored at -20°C until use. Stock solution was further diluted with saline prior to each experiment. The highest concentration of the vehicle in venous perfusion was 0.4 mM or 0.01%.

Adenosine (Adenocor) was obtained from Sanofi-synthelabo (Portugal). Dimethylsulfoxide was obtained from J.T.Baker (Holland). Domperidone was obtained from Sigma-RBI Chemical (Portugal/USA). Dopamine (Medopa) was obtained from Medifar (Portugal). Heparine sodium was obtained from B. Braun Medical (Portugal). Isobutylmethylxanthine was obtained from Sigma-Aldrich (Portugal). SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazole-[4,3-e]-1,2,4-triazolo[1,5-c] pyrimidine) was obtained from Sigma-RBI Chemical (Portugal/USA). Sodium pentobarbitone (Eutasil) was obtained from Sanofi-Veterinária (Miraflares, Algés, Portugal).

Results

Age-related evolution of the body weight

There is an age-related increase in body size in rats between 3 and 24 months. Statistically significant

differences were found between the body weight of 3 months old animals (431.0 ± 7.4 g, $n=30$) and that of older rats. No weight differences were observed between the 12 (618.1 ± 16.9 g) and the 24 (641.0 ± 11.3 g) months old animals.

Effects of aging in resting ventilatory and cardiovascular parameters

The effects of aging on the resting cardioventilatory parameters in anesthetized rats are illustrated in Fig. 1. The basal values of f_R remained constant throughout aging (49.8 ± 1.2 , 52.26 ± 1.5 , and 46.6 ± 1.5 breaths min^{-1} , respectively, in 3, 12, and 24 months old rats). Whereas V_T (8.8 ± 0.3 , 6.0 ± 0.3 , and 7.0 ± 0.4 ml kg^{-1} , respectively, in 3, 12, and 24 months old rats) and V_E (442.5 ± 24.2 , 316.4 ± 12.4 , and 322.9 ± 18.8 ml $\text{min}^{-1} \text{kg}^{-1}$, respectively, in 3, 12, and 24 months old rats), decreased significantly ($p < 0.01$) after 12 months old (Fig. 1a–c).

Basal values of HR and BP at 12 months old were 322.2 ± 9.2 beats min^{-1} and 92.6 ± 5.4 mmHg, respectively. These values were lower than those observed in 3 months old animals but not statistically different than those recorded in older animals (Fig. 1d and e)

Effect of aging on ventilatory responses induced by common carotid occlusions

As previously described (Monteiro and Ribeiro 1989), bilateral CCO during 5, 10, and 15 s induced a time-

dependent excitatory effect on ventilation (Fig. 2) followed by an inhibitory effect caused by reoxygenation, both abolished by carotid sinus nerve section. This excitatory effect on ventilation caused by carotid ischemic stimuli was also present in old rats (Fig. 2). The transient increases in BP observed in young and old animals during CCO (Fig. 2a and b), were similar in both groups. No apparent changes in HR were detected during CCO in the three groups of animals.

Cardioventilatory responses to dopamine

Dose–response curves for the effects of i.c. cumulative bolus injections of dopamine (3–100 nmol) on cardioventilatory parameters in anesthetized and vagotomized rats are shown in Fig. 3. The inhibitor effect of dopamine on ventilation and its slight increase in HR and BP were dose-dependent until approximately 30 nmol and of the same magnitude in both young adults and aged rats. As previously shown by others (Zapata and Zuazo 1980), the bilateral section of the CSN completely abolished the inhibitory effect caused by dopamine on f_R , V_T , and V_E but did not change the increase in BP (not shown). The effect of dopamine on cardioventilatory parameters was also tested in the presence of domperidone, a dopamine D_2 -receptor selective antagonist that does not cross the blood–brain barrier. Domperidone (0.01 – 0.5 mg $\text{kg}^{-1} \text{min}^{-1}$ /2.35–137.5 $\mu\text{mol} \text{kg}^{-1} \text{min}^{-1}$; i.v. infusion) almost totally abolished the depression on V_E induced by dopamine (Fig. 4). The antagonism is dose-dependent, and of the

Fig. 1 Effect of aging on basal values of respiratory frequency (f_R), tidal volume (V_T), respiratory minute volume (V_E), heart rate (HR), and arterial blood pressure (BP) in anesthetized and vagotomized rats ($n=20$). Data represent mean $\pm$ SEM, * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$, Mann–Whitney U test vs 3 months old rats

Fig. 2 Effects of common carotid occlusions (CCO) on respiratory airflow (PulmFI; ml/s) respiratory rate (f_R), tidal volume (V_T or TV), respiratory minute volume (V_E), and arterial blood pressure in anesthetized and vagotomized rats throughout aging ($n=10$). Original recordings obtained for CCO, during

10 s (a) and 15 s (b) in a 3 and a 24 months old rat. c Dose-response curves obtained in six animals: open square 3 months old, close triangle 12 months old, and close square 24 months old; 0% effect corresponds to the values showed in Fig. 1. Data represent mean $\pm$ SEM

same magnitude in 3, 12, and 24 months old rats (Fig. 4a–c). In contrast, domperidone (0.01–0.5 mg kg⁻¹ min⁻¹, i.v.) did not modify the effects of dopamine on BP and HR either in young or aged animals (Fig. 4d and e). The effect of domperidone infusion (0.01–0.1 mg kg⁻¹ min⁻¹) by itself in the absence of exogenous dopamine, on V_E in 3 and 24 months old rats is illustrated in Fig. 5. Domperidone alone caused statistically significant increases in basal V_E in both young and aged animals in a dose-dependent manner, but remarkably the excitatory effect of this D₂-dopamine antagonist on ventilation is significantly ($p<0.01$) attenuated in aged animals (Fig. 5a). The maximal excitatory effect (66.8 $\pm$ 8.1 breaths kg⁻¹ min⁻¹) was achieved with 0.1 mg kg⁻¹ min⁻¹ in young animals. The higher dosage (0.5 mg kg⁻¹ min⁻¹) caused a less pronounced (44.3 $\pm$ 12.9%) effect on ventilation. Domperidone

(0.01 and 0.1 mg kg⁻¹ min⁻¹) by itself did not cause apparent modifications in both HR and BP.

Cardioventilatory responses to adenosine

The results obtained in the experiments performed to investigate whether the excitatory effect of adenosine on ventilation was modified by age are shown in Fig. 6. Dose-response curves for the effects of i.c. cumulative bolus injections of adenosine (3–100 nmol) on f_R , V_T , V_E , HR, and BP were obtained in 3 and 24 months old rats. As expected, adenosine by itself caused excitatory effects on V_E due to increases in both f_R and V_T (Fig. 6a–c). Adenosine 100 nmol increased V_E by 60.9 $\pm$ 2.9 and 55.1 $\pm$ 3.7%, respectively, in 3 and 24 months old rats. The responses in f_R , V_T , and V_E to exogenously administered adenosine were remarkably similar in 3 and in 24 months old rats (Fig. 6a–c). As

Fig. 3 Effects of i.c. cumulative bolus injections of dopamine on respiratory airflow (PulmFI; ml/s), respiratory rate (f_R), tidal volume (V_T), respiratory minute volume (V_E), heart rate (HR), and arterial blood pressure (BP) in anesthetized and vagotomized rats throughout aging. Original recordings obtained for

dopamine 30 nmol: **d** 3 months and **e** 24 months old rats. **a**, **b**, **c**, and **f** dose–response curves obtained in 20 animals: *open square* 3 months old, *close triangle* 12 months old, and *close square* 24 months old; 0% effect corresponds to the values showed in Fig. 1. Data represent mean $\pm$ SEM

previously shown by others (Monteiro and Ribeiro 1987), the respiratory effects of adenosine were abolished by CSN section (not shown). An immediate decrease in the HR and BP remaining in animals after bilateral section of the CSN was also induced by exogenous adenosine. The hypotensive and bradycardic effects of adenosine (100 nmol) were clearly attenuated in 24-month-old rats (Fig. 6d and e).

In a group of experiments, i.c. bolus injections of adenosine were performed during i.v. infusion of an A_{2A} -adenosine receptor antagonist, SCH 58261. The effect of adenosine 100 nmol on V_E was fully abolished by SCH 58261, 20 $\text{ng kg}^{-1} \text{ min}^{-1}$, in both young and old animals (Fig. 7a and b). Figure 7 also depicts that the adenosine A_{2A} -receptors blockade (SCH 58261; 2–20 $\text{ng kg}^{-1} \text{ min}^{-1}$ i.v.) did not prevent the decrease in HR and BP evoked by adenosine in both young and old animals. In contrast, a small potentiating of the bradycardic effect of adenosine by SCH 58261 was observed in old animals (Fig. 7d).

SCH58261 alone in the dose of 2 $\text{ng kg}^{-1} \text{ min}^{-1}$ i.v. significantly ($p > 0.01$) increased V_E (18.5 ± 0.5 and $16.4 \pm 0.8\%$, respectively, in 3 and 24 months old rats), whereas the dose of 20 $\text{ng kg}^{-1} \text{ min}^{-1}$ i.v. did not cause, by itself, appreciable changes in V_E in both 3 and 24 months old rats (Fig. 8).

Administration of the adenosine A_{2A} -receptor antagonist SCH 58261 alone had no effect on HR or BP (Fig. 8).

Effects of age on cAMP levels in CBs in response to different oxygen concentrations

The cAMP levels in CBs isolated from 3, 12, 18, and 24 months old rats incubated in normoxic solutions (20% O_2) are shown in Fig. 9a. No differences were found between cAMP levels in CBs of young and old rats expressed by CB or corrected by the CB weight at different ages (Fig. 9a). The weight of the CBs (μg) changed slightly throughout aging and was: 49 ± 7 (3 months), 46 ± 8 (12 months), 75 ± 12 (18 months), and 70 ± 10 (24 months).

Figure 9b shows the effect of aging on cAMP production induced by different oxygen concentrations applied to the CBs. PO_2 of around 677 mmHg (incubating solutions equilibrated with 95% O_2 /5% CO_2) yields cAMP levels significantly lower than those found when the incubating PO_2 is close to physiological or normoxic (≈ 142 mmHg; incubating solutions equilibrated with 20% O_2 /5% CO_2). In the CB, the normoxic level of cAMP was maintained at lower PO_2 of ≈ 71 (10% O_2) and 35 mmHg (5% O_2).

Fig. 4 Dose–response curves for the effects of cumulative i.c. bolus injections of dopamine on respiratory minute volume (V_E), heart rate (HR), and blood pressure (BP) in anesthetized and vagotomized rats, in the absence and in the presence of i.v. infusion of domperidone. **a** and **d** 3 months old rats, **b** 12 months old rats, and **c** and **e** 24 months old rats. *Open square* in the absence of domperidone ($n=13–20$), in the

presence of i.v. domperidone ($n=6$); *close triangle* 0.01 mg kg⁻¹ min⁻¹; *close diamond* 0.1 mg kg⁻¹ min⁻¹; and *close circle* 0.5 mg kg⁻¹ min⁻¹; 0% increase represents absolute values determined for the 12 s that preceded the injections. Data represent mean±SEM, * $p<0.01$; ** $p<0.001$; *** $p<0.0001$, Mann–Whitney U test compared with *open square*

This pattern and the amount of cAMP production by the carotid bodies did not change with aging.

Discussion

This functional approach of carotid body chemoreceptor activity, which included ventilatory responses to ischemia, pharmacological manipulation of two important carotid body neuromediators and intracellular cAMP accumulation induced by changes in O_2 ,

showed that peripheral control of ventilation is apparently not impaired by aging.

The aging process is characterized by a decline in several physiological functions resulting in a reduction in the ability to maintain homeostasis (Troen 2003). However, there is increasing evidence that several physiological functions are well preserved in aging. Namely, several studies have examined the age-related changes in the ventilatory response to hypoxia and found a maintained ventilatory response throughout aging, suggesting that no alteration in

Fig. 5 Effect of domperidone i.v. by itself on respiratory minute volume (V_E), heart rate (HR), and blood pressure (BP) in anesthetized and vagotomized 3 and 24 months old rats ($n=$

6). Only one dose was tested per animal. Data represent mean±SEM, * $p<0.01$, Mann–Whitney U test comparing 3 vs 24 months old rats

Fig. 6 Effects of i.c. cumulative bolus injections of obtained adenosine on respiratory airflow (PulmFI; ml/s), respiratory rate (f_R), tidal volume (V_T), respiratory minute volume (V_E), heart rate (HR), and arterial blood pressure (BP) in anesthetized and vagotomized rats throughout aging. Original recordings for adenosine 30 nmol: **d** 3 months and **e** 24 months old rats. **a**, **b**,

c, **f**, and **g** dose–response curves obtained in: *open square* 3 months old and *close square* 24 months old ($n=12$); 0% effect correspond to the values showed in Fig. 1. Data represent mean $\pm$ SEM, * $p<0.01$; *** $p<0.0001$, Mann–Whitney U test compared with *open square*

peripheral chemoreception occurred in old rats (Pokorski and Antosiewicz 2010) or humans (Smith et al. 2001; Pokorski et al. 2004; Vovk et al. 2004).

The present work contributes indirectly to knowing the effect of aging in the oxygen-sensing mechanisms, and was focused in the peripheral control of ventilation mediated by the CB.

It was previously described that respiratory frequency decreases with the size increase of the animals and declines in a linear fashion with the age increase in rat (Soulaige et al. 2004). Healthy elderly subjects at rest breathe with a V_E identical to that of younger subjects, but with smaller V_T and higher f_R (Janssens et al. 1999). In our model's experimental conditions, a significant increase in rat weight was found after 12 months of age, but the basal values of f_R remained constant throughout aging. The animals were vagotomized and under the influence of sodium pentobarbitone anesthesia and consequently showed lower

values of f_R (46.6 ± 1.5 breaths min^{-1}). This could explain the absence of the bradypnea found by others in old rats where basal values of f_R were 104.7 ± 3.4 breaths min^{-1} (Soulaige et al. 2004).

It is well known that, in general, all anesthetic regimens cause respiratory and metabolic depression. Anesthesia predominantly depresses respiratory frequency, with little or no effect on V_T , and it has been reported that pentobarbitone depresses V_E by 30% (Schwenke and Cragg 2004). Pentobarbital anesthesia was elected because it produces a steady respiratory baseline (Young 1957) and it does not affect or even potentiate the respiratory reflex reactivity to chemoreceptor stimulation (Douglas et al. 1950). Also, Biscoe and Millar (1968) felt that pentobarbital did not affect carotid chemoreceptor discharge significantly (Biscoe and Millar 1968). Furthermore, some authors found that the magnitude of the V_E response to hypoxia and hypercapnia appears to be unaltered

Fig. 7 Dose–response curves for the effects of cumulative i.c. bolus injections of adenosine on respiratory minute volume (V_E), heart rate (HR), and blood pressure (BP) in anesthetized and vagotomized rats, in the absence and in the presence of SCH 58261 i.v. **a**, **c**, and **e** 3 months old rats; **b**, **d**, and **f** 24 months old rats. *Open square* in the absence of SCH 58261 ($n=12$), in the

presence of i.v. SCH 58261 ($n=6$); *open diamond* $2 \text{ ng kg}^{-1} \text{ min}^{-1}$ and *close diamond* $20 \text{ ng kg}^{-1} \text{ min}^{-1}$; 0% increase represents absolute values determined for the 12 s that preceded the injections of adenosine. Data represent mean $\pm$ SEM, * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$, Mann–Whitney U test compared with *open square*

by pentobarbitone anesthesia (Schwenke and Cragg 2004). The initial anesthetic dose given to old animals was lower ($40 \text{ mg kg}^{-1} \text{ ip}$) than that given to young adults ($60 \text{ mg kg}^{-1} \text{ ip}$), and in general they require fewer anesthetic supplements during the experiments to suppress pain reflexes (see details in the online methods supplement). This difference has been attributed to pharmacokinetic issues in the elderly where the rapid intercompartmental clearance is 30% lower in the old when compared to young subjects,

resulting in higher concentrations and more drugs available for distribution to the brain (Bowie and Slatum 2007). Anyway, no age differences in brain responsiveness were found: no change was detected in the action of GABA_A receptor modulators like pentobarbital (Griffith et al. 2000).

The absence of changes in f_R when the input of the major pulmonary stretch receptors is abolished by vagotomy and CNS activity depressed by the barbiturate, suggest that changes in f_R in the elderly could

Fig. 8 Effect of SCH 58261 alone (*open diamond* 2 and *close diamond* $20 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v., 3 min infusion) on respiratory minute volume (V_E), heart rate (HR), and arterial blood pressure (BP) in anesthetized and vagotomized rats. **a** 3 (*open square*)

and 24 (*close square*) months old rats ($n=6$). Only one dose was tested per animal. Data represent mean $\pm$ SEM, ** $p < 0.001$, Mann–Whitney U test compared with vehicle and between the two doses of SCH 58261

Fig. 9 a Effect of aging on cAMP levels (expressed as picomoles per CB and as picomoles per milligram of tissue) in the CB of rats ($n=12-16$) incubated in normoxic conditions

(20% O₂). **b** Comparison between the cAMP levels in the CBs of 3 ($n=14-18$) and 24 ($n=10-12$) months old rats incubated with different oxygen concentrations. Data are mean $\pm$ SEM

not be attributed to an impairment of the CB peripheral drive.

Wistar rats exhibited, in the present conditions, a reduction of tidal volume with aging. Although some authors did not find tidal volume to decrease with advancing age (Soulaige et al. 2004), an age-related decrease was commonly observed in rats (Nagase et al. 1994) and humans (Janssens et al. 1999) with advancing age, and has been linked to alterations of the mechanical properties of lung and thorax (Chan and Welsh 1998).

The cardiovascular effects associated with aging are: a striking attenuation in the cardiac frequency in humans (Kronenberg and Drage 1973); no significant effect of age on resting HR of rats (Gordon 2008); and increase in blood pressure in both rats (Di Nardo et al. 2009) and humans (Fleg et al. 1995). In the present work, mean arterial blood pressure was lower in 24 months old rats than in young adults. This finding could be attributed to the predominant effect of anesthetic-mediated central cardiovascular depression (Schwenke and Cragg 2004) rather than the arterial dysfunction in aging. It is known that the risk of cardiovascular depression induced by barbiturates is higher in the elderly (Schwenke and Cragg 2004) and compatible with the lower values for BP found in the present work, despite the lower doses of anesthetic were needed to abolish sensitivity in old animals.

There is a consensual body of evidence about the changes in the morphology of the CB throughout aging: increase in extracellular matrix, reduction in number and volume of type I cells, and in the volume and density of mitochondria compared with young CB (Hurst et al. 1985; Pokorski et al. 2004; Conde et

al. 2006). These morphological findings do not apparently concur with the absence of functional impairment. However, the increase in the number of type II stem cells could contribute towards the maintenance of CB function (Hurst et al. 1985; Porzionato et al. 2005). The present work supports previous findings in humans—absence of alterations in the peripheral control of ventilatory responses to hypoxia (Pokorski et al. 2004; Vovk et al. 2004), providing evidence that structural and neurochemical changes caused by aging in the carotid body are further compensated. The finding that changes in cAMP content at the CB in response to O₂ concentrations did not change with aging, suggests that the compensatory mechanism could occur within the CB as a whole. These results contrast with the reduction in carotid sinus nerve discharges in response to hypoxia observed in old rats (Conde et al. 2006). It is known that the glossopharyngeal nerve has, in addition to carotid sinus nerve afferent fibers, a parallel autonomic parasympathetic efferent pathway sensitive to hypoxia that is the source of CB inhibition (Campanucci et al. 2006). We can speculate that the reduction in CSN discharges found in old animals (Conde et al. 2006) could mainly be due to an impairment of the efferent fiber activity without changes in the chemosensory excitatory afferent pathway. Similarly, it was shown in humans that aging impairs the autonomic responses to pain but does not modify nociceptive perception (Hajduczuk et al. 1991).

Hyperventilation induced by bilateral brief (<15 s) occlusions of the common carotid arteries are abolished by carotid sinus nerve section (Monteiro and

Ribeiro 1989), constituting an alternative model for hypoxia in order to study CB chemosensory activation *in vivo*. In the present work, the cardiorespiratory reflexes induced by carotid ischemia were similar in young and old rats. Previous work has addressed the impairment of carotid ischemia on cerebral oxidative balance caused by permanent ligation of the right carotid artery (Macri et al. 2010) in old animals. These are long-term ischemia and the authors did not address the effects of the ventilatory reflexes induced by carotid ischemia in aged animals, shown for the first time in the present work. Carotid sinus baroreflex function throughout aging has never been studied using this model but the persistency of carotid baroreflex function in advanced age rats was previously described in anesthetized Fisher rats (Wei et al. 1986). In humans, there is no difference in carotid body baroreflexes throughout aging (Fiocchi et al. 1985; Shi et al. 1996).

Another aspect to consider in our discussion pertains to the age-dependent variations in cAMP levels and responses to hypoxia. Aging did not cause appreciable changes in cAMP levels in the CB. The normoxic cAMP levels do not differ in the four ages studied, and the pattern of cAMP production in response to different O₂ concentrations remained almost unchanged in old animals. The cAMP levels found in the rat CB in the present study, expressed in unit weight, are nearly identical to the values reported in the rabbit CB (Perez-Garcia et al. 1990; Chen et al. 1997). At all ages, cAMP levels were maximal in normoxic conditions. Compared to normoxia, hyperoxia caused a decrease in cAMP in the CB in all ages. Adenosine effects via A₂ receptors, which are known to be expressed in the CB (Gauda 2002), can represent one of the mechanisms maintaining high cAMP levels in hypoxia. It is worth noting that the release of adenosine in the CB is maximal at mild levels of hypoxia (10%O₂), decreasing with higher intensities of hypoxic stimulation and that the most intense hypoxia used in this study does not increase the release of adenosine in the SCG or CA (Conde and Monteiro 2004). Of course adenosine is not the only modulator of cAMP levels in the CB, as it is well known that hypoxia also increases the release of many other neurotransmitters, particularly dopamine (Vicario et al. 2000). It is also known that the CB expresses high levels of D₂ dopamine receptors (Gauda 2002) which are negatively coupled to

adenylate cyclase (Kebabian and Calne 1979). In fact, it would appear that the much higher rate of dopamine release in the rat vs. the rabbit CB in hypoxia (Vicario et al. 2000) could explain the inability of hypoxia to increase the cAMP levels in the rat CB above those found in normoxia, as it has been previously observed in the rabbit (Cachero et al. 1996). It should be mentioned that Mir et al. (Mir et al. 1983), in their pioneer study, also found that 5% O₂ administered *in vivo* did not significantly modify cAMP levels in the rat CB.

The absence of significant changes in cAMP production in old animals is not incompatible with individual changes in adenylate cyclase activity and/or metabotropic receptors but implies that if individual changes occurred the overall response is maintained.

The effects of aging in cAMP accumulation in other preparations show impairment of the activity of the catalytic subunit of adenylate cyclase in rats (Kilts et al. 2002) and in the heart of >60-year-old humans (Brodde et al. 1995). However, it has been described that the maximal ability of forskolin to increase cAMP in the adrenal medulla and liver is enhanced in 24-month-old rats (Tumer et al. 1996).

If a reduction in both A₂-adenosine (positively coupled to adenylate cyclase) and D₂-dopamine (negatively coupled to adenylate cyclase) receptor-mediated responses coexist in the CB, then compensatory effects in cAMP production can take place. In fact, the results obtained in the present work with exogenous adenosine and dopamine as well as manipulating their endogenous effects through antagonists showed that neither adenosine or dopamine receptors are significantly impaired in old animals. These dopamine and adenosine effects were mediated through carotid body chemoreceptors, seeing as these actions disappeared after bilateral section of the carotid sinus nerves.

An additional interest to test the effects of dopamine and adenosine in the present model is their specific therapeutic indications in clinical practice: shock and supraventricular tachycardia, respectively. These clinical situations are associated with heart failure, more prevalent in the elderly, and the cardiorespiratory effects of exogenous dopamine and adenosine in aged humans or animals have never been investigated.

Dopamine has been shown to impair the ventilatory drive in response to hypoxemia and depress minute ventilation and oxygen saturation in heart failure patients, even when they are breathing room air (van de Borne et al. 1998). The present work provides evidence that these undesirable effects should be of the same magnitude in aged subjects.

Domperidone is a D_2 -selective antagonist that does not cross the blood–brain barrier (Baudry et al. 1979) and, administered alone, it increases basal V_E as described in previous work (Lahiri et al. 1984; Gamboa et al. 2003). We have found statistically significant differences between 3 and 24 months old rats. This means that the inhibitory basal tonus of dopamine at the CB activity is less marked in old rats, probably resulting from a reduction in the number of receptors throughout aging. This is not an unexpected finding considering that it has previously been found that the density of striatal D_2 -receptors is significantly reduced in aged rats between 30% and 80% depending on the study (Marshall and Joyce 1988; Petkov et al. 1988; Han et al. 1989; Popoli et al. 1998). However, in the CB this reduction is not enough to reduce the effect of exogenous dopamine which has the same magnitude in old rats and controls.

The BP response triggered by dopamine, a short-lived rise followed to a return to baseline, occurred independent of the carotid sinus nerve afferentation and blockade of dopamine D_2 -receptors. This result warrants that the prompt hypertensive response evoked by dopamine challenge apparently occurs beyond the baroreceptor afferentation and may depend on D_1 -dopamine receptors at the heart and/or adrenoceptors.

Despite its dyspnoeic effect mediated by chemoreceptor activation (Burki et al. 2005), adenosine is a clinically useful tool to treat supraventricular tachycardia (Biaggioni et al. 1987; Riccardi et al. 2008), a common cardiac rhythm disturbance more frequently observed in the elderly (Medi et al. 2009). However, studies about this effect on aged subjects are yet to be made. Although the present work was not focused in the crono/dromo and batmotropic effects of adenosine, an interesting finding is the lesser bradycardic effect observed during its exogenous application in aged animals, suggesting that the efficacy of adenosine to revert supraventricular tachycardia could be attenuated in the elderly. This could be attributed to a reduction in the density of adenosine receptors in the

aged heart and may also involve an age-related reduction in the intrinsic ability of nodal tissue to respond to adenosine receptor activation (Hinschen et al. 2001). Some authors find an absence of changes in A_1 -receptors density and G alpha protein levels but an adenosine A_1 -receptor function in rat ventricles to decrease with age and this was related to a reduction in the coupling between adenosine A_1 -receptor and their G proteins (Cai et al. 1997).

The excitatory effects of adenosine found in old rats suggest that the A_2 -adenosine receptors in the carotid body are well preserved and that the chest discomfort caused by its exogenous administration will remain in the elderly. The excitatory effects obtained with low doses of SCH 58261 by itself, an adenosine A_{2A} -antagonist that crosses the blood–brain barrier (El Yacoubi et al. 2000), are in agreement with the presence of A_{2A} receptors with inhibitory effects on the control of breathing (Mayer et al. 2006) and that central inhibitory control is apparently more relevant in basal conditions than the excitatory effects peripherally mediated. Therefore, low doses of A_{2A} antagonists that cross BBB (like SCH 58261) could be useful to treat central mediated ventilatory depressions without major actions in the cardiovascular system because the dose that antagonize the ventilatory effects did not modify BP and HR.

In short, we found that carotid body peripheral control of ventilation is not impaired with aging. Endogenous dopaminergic inhibitory tonus in the CB is slightly reduced. However, the decrease in ventilation caused by exogenous dopamine keeps visible in aged animals and its use should be taken into consideration in critical care situations. The excitatory effect of exogenous adenosine mediated by A_{2A} receptors in the carotid body chemosensors was not modified in aging. In contrast, the bradycardic effect of exogenous adenosine mediated by A_1 receptors is attenuated in old animals. These findings reduce the clinical interest of adenosine's use to revert supraventricular tachycardia in aged people. The maintained carotid body function throughout aging confirms this organ as a valuable model of successful aging.

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References

- Alcayaga J, Iturriaga R, Zapata P (1986) Carotid body chemoreceptor excitation produced by carotid occlusion. *Acta Physiol Pharmacol Latinoam* 36:199–215
- Batuca JR, Monteiro TC, Monteiro EC (2003) Contribution of dopamine D2 receptors for the cAMP levels at the carotid body. *Adv Exp Med Biol* 536:367–373
- Baudry M, Martres MP, Schwartz JC (1979) 3H-Domperidone: a selective ligand for dopamine receptors. *Naunyn-Schmiedeberg Arch Pharmacol* 308:231–237
- Biaggioni I, Olafsson B, Robertson RM, Hollister AS, Robertson D (1987) Cardiovascular and respiratory effects of adenosine in conscious man. Evidence for chemoreceptor activation. *Circ Res* 61:779–786
- Biscoe TJ, Millar RA (1968) Effects of inhalation anaesthetics on carotid body chemoreceptor activity. *Br J Anaesth* 40:2–12
- Bowie MW, Slatum PW (2007) Pharmacodynamics in older adults: a review. *Am J Geriatr Pharmacother* 5:263–303
- Brodde OE, Zerkowski HR, Schranz D, Broede-Sitz A, Michel-Reher M, Schafer-Beisenbusch E, Piotrowski JA, Oelert H (1995) Age-dependent changes in the beta-adrenoceptor-G-protein(s)-adenylyl cyclase system in human right atrium. *J Cardiovasc Pharmacol* 26:20–26
- Burki NK, Dale WJ, Lee LY (2005) Intravenous adenosine and dyspnea in humans. *J Appl Physiol* 98:180–185
- Cachero TG, Rigual R, Rocher A, Gonzalez C (1996) Cholera and pertussis toxins reveal multiple regulation of cAMP levels in the rabbit carotid body. *Eur J Neurosci* 8:2320–2327
- Cai G, Wang HY, Gao E, Horwitz J, Snyder DL, Pelleg A, Roberts J, Friedman E (1997) Reduced adenosine A1 receptor and G alpha protein coupling in rat ventricular myocardium during aging. *Circ Res* 81:1065–1071
- Campanucci VA, Zhang M, Vollmer C, Nurse CA (2006) Expression of multiple P2X receptors by glossopharyngeal neurons projecting to rat carotid body O2-chemoreceptors: role in nitric oxide-mediated efferent inhibition. *J Neurosci* 26:9482–9493
- Chan ED, Welsh CH (1998) Geriatric respiratory medicine. *Chest* 114:1704–1733
- Chen J, Dinger B, Fidone SJ (1997) cAMP production in rabbit carotid body: role of adenosine. *J Appl Physiol* 82:1771–1775
- Conde SV, Monteiro EC (2004) Hypoxia induces adenosine release from the rat carotid body. *J Neurochem* 89:1148–1156
- Conde SV, Obeso A, Rigual R, Monteiro EC, Gonzalez C (2006) Function of the rat carotid body chemoreceptors in ageing. *J Neurochem* 99:711–723
- Di Nardo F, Burattini R, Cogo CE, Faelli E, Ruggeri P (2009) Age-related analysis of insulin resistance, body weight and arterial pressure in the Zucker fatty rat. *Exp Physiol* 94:162–168
- Douglas WW, Innes IR, Kosterlitz HW (1950) The vasomotor responses due to electrical stimulation of the sinus and vagus nerves of the cat and their modification by large doses of sodium pentobarbital (nembutal). *J Physiol* 111:215–230
- El Yacoubi M, Ledent C, Parmentier M, Costentin J, Vaugeois JM (2000) The anxiogenic-like effect of caffeine in two experimental procedures measuring anxiety in the mouse is not shared by selective A(2A) adenosine receptor antagonists. *Psychopharmacology (Berl)* 148:153–163
- Fiocchi R, Fagard R, Vanhees L, Grauwels R, Amery A (1985) Carotid baroreflex sensitivity and physical fitness in cycling tourists. *Eur J Appl Physiol Occup Physiol* 54:461–465
- Fleg JL, O'Connor F, Gerstenblith G, Becker LC, Clulow J, Schulman SP, Lakatta EG (1995) Impact of age on the cardiovascular response to dynamic upright exercise in healthy men and women. *J Appl Physiol* 78:890–900
- Gamboa J, Macarlupu JL, Rivera-Chira M, Monge C, Leon-Velarde F (2003) Effect of domperidone on ventilation and polycythemia after 5 weeks of chronic hypoxia in rats. *Respir Physiol Neurobiol* 135:1–8
- Gauda EB (2002) Gene expression in peripheral arterial chemoreceptors. *Microsc Res Tech* 59:153–167
- Gonzalez C, Almaraz L, Obeso A, Rigual R (1994) Carotid body chemoreceptors: from natural stimuli to sensory discharges. *Physiol Rev* 74:829–898
- Gordon CJ (2008) Cardiac and thermal homeostasis in the aging Brown Norway rat. *J Gerontol A Biol Sci Med Sci* 63:1307–1313
- Griffith WH, Jasek MC, Bain SH, Murchison D (2000) Modification of ion channels and calcium homeostasis of basal forebrain neurons during aging. *Behav Brain Res* 115:219–233
- Hajduczuk G, Chapple MW, Johnson SL, Abboud FM (1991) Increase in sympathetic activity with age. I. Role of impairment of arterial baroreflexes. *Am J Physiol* 260: H1113–H1120
- Han Z, Kuyatt BL, Kochman KA, DeSouza EB, Roth GS (1989) Effect of aging on concentrations of D2-receptor-containing neurons in the rat striatum. *Brain Res* 498:299–307
- Hinschen AK, Rose-Meyer RB, Headrick JP (2001) Age-related changes in adenosine-mediated relaxation of coronary and aortic smooth muscle. *Am J Physiol Heart Circ Physiol* 280:H2380–H2389
- Hurst G, Heath D, Smith P (1985) Histological changes associated with ageing of the human carotid body. *J Pathol* 147:181–187
- Iturriaga R, Alcayaga J (2004) Neurotransmission in the carotid body: transmitters and modulators between glomus cells and petrosal ganglion nerve terminals. *Brain Res Brain Res Rev* 47:46–53
- Janssens JP, Pache JC, Nicod LP (1999) Physiological changes in respiratory function associated with ageing. *Eur Respir J* 13:197–205
- Kebabian JW, Calne DB (1979) Multiple receptors for dopamine. *Nature* 277:93–96
- Kilts JD, Akazawa T, Richardson MD, Kwatra MM (2002) Age increases cardiac $\text{G}\alpha_{i2}$ expression, resulting in enhanced coupling to G protein-coupled receptors. *J Biol Chem* 277:31257–31262
- Kronenberg RS, Drage CW (1973) Attenuation of the ventilatory and heart rate responses to hypoxia and hypercapnia with aging in normal men. *J Clin Invest* 52:1812–1819

- Lahiri S, Smatresk N, Pokorski M, Barnard P, Mokashi A, McGregor KH (1984) Dopaminergic efferent inhibition of carotid body chemoreceptors in chronically hypoxic cats. *Am J Physiol* 247:R24–R28
- Macri MA, D'Alessandro N, Di GC, Di IP, Di LS, Giuliani P, Esposito E, Pokorski M (2010) Region-specific effects on brain metabolites of hypoxia and hyperoxia overlaid on cerebral ischemia in young and old rats: a quantitative proton magnetic resonance spectroscopy study. *J Biomed Sci* 17:14
- Marek W, Muckenhoff K, Prabhakar NR (2008) Significance of pulmonary vagal afferents for respiratory muscle activity in the cat. *J Physiol Pharmacol* 59(Suppl 6):407–420
- Marshall JF, Joyce JN (1988) Basal ganglia dopamine receptor autoradiography and age-related movement disorders. *Ann N Y Acad Sci* 515:215–225
- Mayer CA, Haxhiu MA, Martin RJ, Wilson CG (2006) Adenosine A2A receptors mediate GABAergic inhibition of respiration in immature rats. *J Appl Physiol* 100:91–97
- McQueen DS, Ribeiro JA (1986) Pharmacological characterization of the receptor involved in chemoexcitation induced by adenosine. *Br J Pharmacol* 88:615–620
- McQueen DS, Bond SM, Moores C, Chessell I, Humphrey PP, Dowd E (1998) Activation of P2X receptors for adenosine triphosphate evokes cardiorespiratory reflexes in anaesthetized rats. *J Physiol* 507(Pt 3):843–855
- Medi C, Kalman JM, Freedman SB (2009) Supraventricular tachycardia. *Med J Aust* 190:255–260
- Mir AK, Pallot DJ, Nahorski SR (1983) Biogenic amine-stimulated cyclic adenosine-3', 5'-monophosphate formation in the rat carotid body. *J Neurochem* 41:663–669
- Monteiro EC, Ribeiro JA (1987) Ventilatory effects of adenosine mediated by carotid body chemoreceptors in the rat. *Naunyn-Schmiedeberg Arch Pharmacol* 335:143–148
- Monteiro EC, Ribeiro JA (1989) Inhibition by 1, 3-dipropyl-8 (p-sulfophenyl)xanthine of the respiratory stimulation induced by common carotid occlusion in rats. *Life Sci* 45:939–945
- Nagase T, Fukuchi Y, Teramoto S, Matsuse T, Orimo H (1994) Mechanical interdependence in relation to age: effects of lung volume on airway resistance in rats. *J Appl Physiol* 77:1172–1177
- Nishino T, Lahiri S (1981) Effects of dopamine on chemoreflexes in breathing. *J Appl Physiol* 50:892–897
- Perez-Garcia MT, Almaraz L, Gonzalez C (1990) Effects of different types of stimulation on cyclic AMP content in the rabbit carotid body: functional significance. *J Neurochem* 55:1287–1293
- Petkov VD, Petkov VV, Stancheva SL (1988) Age-related changes in brain neurotransmission. *Gerontology* 34:14–21
- Pokorski M, Antosiewicz J (2010) Alterations in the hypoxic ventilatory response with advancing age in awake rats. *J Physiol Pharmacol* 61:227–232
- Pokorski M, Walski M, Dymecka A, Marczak M (2004) The aging carotid body. *J Physiol Pharmacol* 55(Suppl 3):107–113
- Popoli P, Betto P, Rimondini R, Reggio R, Pezzola A, Ricciarello G, Fuxe K, Ferre S (1998) Age-related alteration of the adenosine/dopamine balance in the rat striatum. *Brain Res* 795:297–300
- Porzionato A, Macchi V, Guidolin D, Parenti A, Ferrara SD, De CR (2005) Histopathology of carotid body in heroin addiction. Possible chemosensitive impairment. *Histopathology* 46:296–306
- Riccardi A, Arboscello E, Ghinatti M, Minuto P, Lerza R (2008) Adenosine in the treatment of supraventricular tachycardia: 5 years of experience (2002–2006). *Am J Emerg Med* 26:879–882
- Schwenke DO, Cragg PA (2004) Comparison of the depressive effects of four anesthetic regimens on ventilatory and cardiovascular variables in the guinea pig. *Comp Med* 54:77–85
- Shi X, Gallagher KM, Welch-O'Connor RM, Foresman BH (1996) Arterial and cardiopulmonary baroreflexes in 60- to 69- vs. 18- to 36-yr-old humans. *J Appl Physiol* 80:1903–1910
- Smith WD, Poulin MJ, Paterson DH, Cunningham DA (2001) Dynamic ventilatory response to acute isocapnic hypoxia in septuagenarians. *Exp Physiol* 86:117–126
- Soulaie C, Pequignot JM, Perrin D (2004) Breathing pattern and hypoxic sensitivity during ageing in a new model of obesity-resistant rat. *Respir Physiol Neurobiol* 144:45–57
- Troen BR (2003) The biology of aging. *Mt Sinai J Med* 70:3–22
- Tumer N, Sego RL, Scarpace PJ (1996) Atypical pattern of adenylyl cyclase activity in the adrenal medulla with age. *Exp Gerontol* 31:571–576
- van de Borne P, Oren R, Somers VK (1998) Dopamine depresses minute ventilation in patients with heart failure. *Circulation* 98:126–131
- Vicario I, Rigual R, Obeso A, Gonzalez C (2000) Characterization of the synthesis and release of catecholamine in the rat carotid body in vitro. *Am J Physiol Cell Physiol* 278:C490–C499
- Vovk A, Smith WD, Paterson ND, Cunningham DA, Paterson DH (2004) Peripheral chemoreceptor control of ventilation following sustained hypoxia in young and older adult humans. *Exp Physiol* 89:647–656
- Wei JY, Mendelowitz D, Anastasi N, Rowe JW (1986) Maintenance of carotid baroreflex function in advanced age in the rat. *Am J Physiol* 250:R1047–R1051
- Young IM (1957) Some observations on the mechanism of adrenaline hyperpnoea. *J Physiol* 137:374–395
- Zapata P, Zuazo A (1980) Respiratory effects of dopamine-induced inhibition of chemosensory inflow. *Respir Physiol* 40:79–92