Does Ageing Modify Ventilatory Responses to Dopamine in Anaesthetised Rats Breathing Spontaneously?

T.C. Monteiro, A. Obeso, C. Gonzalez and E.C. Monteiro

Abstract Dopamine has been widely used in humans in the management of cardiocirculatory shock, and its inhibitory effect on ventilation has received particular attention in clinical situations more prevalent in the elderly. Dopamine has been extensively studied at the carotid body in adult animals but little is known in aged animals. We investigated the ventilatory responses caused by dopamine in 3 and 24 months old rats.

Cumulative intracarotid bolus injections of dopamine were performed in anaesthetised and vagotomised rats, in the absence and in the presence of i.v. infusions of domperidone (23.5–1175 nmol Kg^{-1} min^{-1}). Airflow (V), tidal volume (V_T), respiratory rate (f), arterial blood pressure and heart rate were monitored and respiratory minute volume (V_E) calculated. Basal values of V_E were lower in 24 months rats (322.9±18.8 mL Kg^{-1} min^{-1}) than in 3 months old rats (442.5±24.2 mL Kg^{-1} min^{-1}), mainly due to reductions in V_T. The dose-dependent decreases caused by dopamine (3–100 nmol) in V_T, f and V_E, were totally prevented by section of the carotid sinus nerve and were not modified by ageing. The maximal % antagonism of the inhibitory effect of dopamine on V_E caused by domperidone was similar in both 3 (74.6±2.7) and 24 (70.7±0.8) months old rats. Domperidone alone, increased basal V_E by 59.6±16.6 mL min^{-1} Kg^{-1}, and by 11.8±1.2 mL min^{-1} Kg^{-1}, respectively in 3 and 24 months old rats ($p<0.01$).

The inhibitory basal tonus caused by dopamine in ventilation was reduced in aged rats, although the decrease in V_E caused by its exogenous administration remained unchanged.

Keywords Ageing · Dopamine breathing · Domperidone · Respiratory frequency · Tidal volume · Respiratory minute volume

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1 Introduction

Ventilatory responses to hypoxia are attenuated with ageing (Fukuda, 1992; Guenard, 1998; Peterson et al., 1981; Chapman and Cherniack, 1987; Janssens et al., 1999) and the measurement in humans of the respiratory drive suggests that there is an age-related decline in the ability to integrate information received from peripheral and central chemoreceptors (Peterson et al., 1981). It is also known that ageing attenuates the increase in carotid sinus nerve afferent activity in response to hypoxia (Conde et al., 2006).

Carotid body chemosensitivity has been extensively studied in animals assessing its dopaminergic function (for a review see Gonzalez et al., 1994). It is known that in aged rats carotid body exhibits a high catecholamine content and turnover time, no changes on the release of dopamine under normoxic conditions but a diminished responsiveness to hypoxic stimuli to evoke the release of this biogenic amine (Conde et al., 2006).

Exogenous dopamine has been widely used in humans to improve renal and cardiovascular function in the management of cardiocirculatory shock, but its inhibitory effect on ventilation could be deleterious especially when dopamine is used in hypoxic patients, in patients with tenuous cardiorespiratory status, or in patients being weaned off ventilatory support (Van de Borne et al., 1998). The prevalence of these limitations is higher in the elderly but the effects of ageing on the ventilatory responses to exogenous administration of dopamine have never been investigated.

In the present work we compared the ventilatory responses induced by exogenous dopamine and by its non-specific D_2 antagonist (Laduron and Leysen, 1979) domperidone, in 3 and 24 months old rats breathing spontaneously.

2 Methods

2.1 Animals and Surgical Procedures

Experiments were performed on male Wistar rats aged 3 months (360–480 g, body weight), and 24 months (500–900 g, body weight). All along their lifespan, the animals were kept in the vivarium of our university, in an air-conditioned room at 21±1°C, 55±10% humity, with a 12:12 h light-dark cycle, and with food and water available ad libitum and experiments were carried out in accordance with the Portuguese regulations for the protection of the animals.

The animals were anaesthetised by a single intraperitoneal injection of sodium pentobarbitone (60 mg Kg^{-1}), supplemented intravenously with 10% of the starting dose as necessary. They were placed supine and breathed spontaneously room air. The trachea was exposed in the neck, sectioned below the larynnx and a pneumotachographic sensor was connected to the distal end of the tracheostomy tubing. Four cannulations were performed under a dissection microscope: the right femoral artery for systemic arterial blood pressure measurement; the right femoral vein for

pharmacological infusions (domperidone at a rate of 0.5 ml min^{-1} during 3 min); the left femoral vein for anaesthesic supplements; and the right external carotid for pharmacological bolus (dopamine i.c. in a volume of 0.1 ml, washed in with 0.2 ml 0.9% aqueous sodium chloride). Body temperature was maintained close to 37°C using a heated underblanket governed by a rectal thermistor probe.

Respiratory flow was measured by a HSE-pneumotachometer PTM type, a differential pressure transducer (model DP 45-14 Validyne Engineering, North-Northridge, CA) and a pressure amplifier (Plugsys Housings, model 603, HSE-HA GmgH). Blood pressure was measureed with a pressure transducer (model Isotec, HSE-HA GmgH) and a pressure amplifier (Plugsys Housings, model 603, HSE-HA GmgH).

We used HSE-Harvard Pulmodyn® W software for data acquisition (system for respiratory studies): signals such as pulmonary airflow, tidal volume (V_T), arterial pressure (BP) and O_2 partial pressure (pO_2) ; and parameters that can be derived from these signals e.g., respiratory rate (f), V_T, heart rate (HR) and mean value of blood pressure, were recorded continuously during experiments. Respiratory minute volume (V_E) was calculated as the product of V_T and f.

The intervals between dopamine injections were at least 5 min. Two cumulative dose-response curves for dopamine (i.c.) were performed in each rat: one with the simultaneous infusion of 0.9% aqueous sodium chloride, and the other after 3 min of i.v. infusion of domperidone. Only one dose of domperidone was tested per animal. Control values for V_T, f, BP and HR correspond to the mean value measured in a period of 25 s immediately before drug administration. After drug i.c. administration the values of V_T, f, BP and HR, were taken as the maximal effects measured during the period of 25 s that followed the injections, and were compared with dose measured during the control. The maximal effects induced by i.c. injections of dopamine always occurred in the first 25 s that followed the end of the injections.

2.2 Drugs

Doses of all drugs were calculated on the basis of salt weight. Dopamine was prepared in 0.9% w/v aqueous sodium chloride solution, and domperidone was prepared in 0.1 N HCl. The drugs used were: sodium pentobarbitone (Eutasil-Sanofi Veterinária), sodium heparin and saline (B. Braun), Dopamine (Medopa-Medinfar), and domperidone (Sigma-Aldrich).

2.3 Statistics

Data are expressed as mean ±S.E.M. and compared for statistical significance using a two tails Student t-test for unpaired data. Significance level was established at $P < 0.05$. Models for analysis were developed using GraphPad Prism software (Version 4.03).

3 Results

Basal values of V_E were lower in 24 months old rats than in 3 months old rats, mainly due to reductions in V_T (Table 1). The dose-dependent decreases caused by dopamine (3–100 nmol) in V_T, f and V_E, were totally prevented by section of the carotid sinus nerve (Fig. 1) and were not modified by ageing (Fig. 2).

Table 1 Comparison of basal ventilatory parameters in 3 and 24 months old rats

	3 months old	24 months old	p value
V_T (ml·Kg^{-1})	8.78 ± 0.34	7.02 ± 0.39	0.0029
f (breaths·min^{-1})	49.75 ± 1.20	46.57 ± 1.54	0.0213
V_E (ml·min-Kg^{-1})	442.50 ± 24.23	322.90 ± 18.83	0.0008

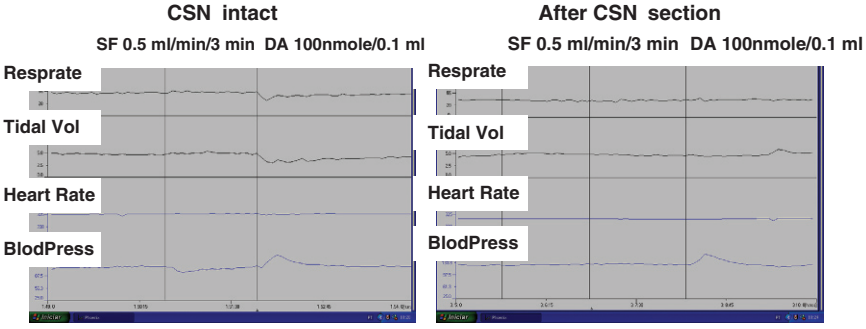

Fig. 1 Effect of i.c. injections of dopamine (100 nmol) on respiratory rate, tidal volume (V_T), heart rate (HR) and mean blood pressure (BP) in a 3 months old rat, anaesthetised and vagotomised, before and after bilateral section of the carotid sinus nerves (CSN)

The maximal % antagonism of the inhibitory effect of dopamine on V_E caused by domperidone was similar in both 3 (74.6±2.7), and 24 (70.7±0.8) months old rats. Domperidone alone (1175 nmol Kg^{-1} min^{-1}), increased basal V_E by 59.6±16.6 mL

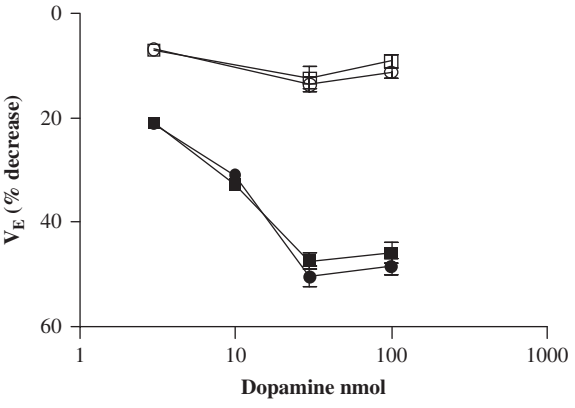

Fig. 2 Comparison between the effects of i.c. injections of dopamine on respiratory minute volume in 3 (■□) and 24 (●○) months old rats in the absence (filled symbols) and during (open symbols) i.v. infusion of domperidone (1175 nmol Kg^{-1} min^{-1})

$min^{-1}\,Kg^{-1}$, and by $11.8\pm1.2\,mL\,min^{-1}\,Kg^{-1}$, respectively in 3 and 24 months old rats ($p<0.01$).

4 Discussion

In this model of vagotomised anaesthetised Wistar rats, ageing decreased ventilation mainly due to a decrease in V_T. The inhibitory basal dopaminergic tonus in ventilation was attenuated in aged animals, but ageing did not modify the effect of exogenous dopamine.

The reduction in resting breathing with ageing in Wistar rats is consensual although some differences between awake and unrestrained rats and vagotomised anaesthetised animals are apparent. In the former case only changes in frequency are evident (Soulage et al., 2004) in contrast with more pronounced reductions in V_T observed in the present work. These results could be linked to the fact that age-related changes in mechanical properties of the lung and thorax become more visible after removing the control of the vagus nerve upon the respiratory frequency. We have used vagotomised animals in the present work because we were particularly interested in the ventilatory effects of dopamine mediated by the carotid body chemoreceptors.

The inhibitory effect of exogenous dopamine on ventilation is mediated by D_2 receptors present at the carotid body chemoreceptors (Mir et al., 1984) and is known since the seventies in both animals (Cardenas and Zapata, 1981) and humans (Welsh et al., 1978). The effects of domperidone by itself on ventilation were attributed to the antagonism of dopamine receptors at the carotid body activated by the endogenous amine, because domperidone does not cross the blood brain barrier and in the doses used in the present work almost totally block the effects of exogenously applied dopamine. These results agree with those previously found by others (Zapata and Torrealba, 1984). The efficacy of domperidone blocking the effects of dopamine on ventilation also indicate that in the range of doses herein tested, the ventilatory effects of dopamine were due exclusively to activation of D_2 receptors and that the involvement of adrenoceptors can be excluded.

The decrease in the excitatory effects of domperidone observed in 24 months old rats could be caused by both a smaller release of dopamine in normoxic conditions and/or a reduction in the number of active dopamine receptors. The absence of age-related changes in the release of dopamine in normoxic conditions in the carotid body in vitro (Conde et al., 2006) is more consistent with an age-related change in dopaminergic D_2 receptors at the carotid body. However, further experiments are needed to exclude any interpretation because the O_2 conditions of this in vivo animal model, can not be exactly extrapolated to those in vitro (Conde et al., 2006) where ageing reduces catecholamine release induced by hypoxia (2 and 5%O_2) in the carotid body. The finding that venous blood dopamine concentrations are higher in healthy sexagenarian than in young alpinists at rest (Serebrovskaya et al., 2000), corroborates the hypothesis that down-regulation of dopamine receptors at the carotid body could occur in the elderly. Age-related impairment of dopamine receptors

have been extensively described in the striatum (for a review sees e.g. Roth and Joseph, 1994).

Although the inhibitory effects of dopamine on ventilation were exclusively mediated by the peripheral chemoreceptors (were abolished by bilateral section of the CSN and dopamine does not cross the blood brain barrier), the absence of differences between the effects of exogenous dopamine in young and aged rats can not necessarily mean that carotid body function is well preserved in ageing. The results of morphological studies show degenerative changes developing with age in the ultrastructure of carotid bodies (Di Giulio et al., 2003; Conde et al., 2006) and carotid sinus nerve activity in response to hypoxia is clearly attenuated in old animals (Conde et al., 2006). The reduction in the excitatory effects of domperidone by itself, found in the present work, also suggests a decrease in carotid body dopaminergic function probably attributed to dopamine receptors desensitization, an effect that can be surpassed by the high doses of dopamine administered intravenously. Since dopamine has mainly inhibitory actions in the peripheral control of breathing, a decrease in endogenous dopaminergic activity at the carotid body with ageing could be considered as a compensatory mechanism to preserve O_2 supply. In addition, ageing by itself did not seem to increase the risk of ventilatory depression caused by the clinical use of dopamine.

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