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Effects of selective carotid body stimulation with adenosine in conscious humans

Running title: Selective carotid body stimulation in humans

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Total number of words: ....

Key words: Adenosine, Carotid body, Stimulation, Chemoreflex, Peripheral Chemoreceptors

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KEY POINTS:

- In conscious humans, excitation of peripheral chemoreceptors with systemic hypoxia causes hyperventilation, hypertension and tachycardia. However, the contribution of particular chemosensory areas (carotid vs. aortic bodies) to this response is unclear.

- We showed that direct and selective unilateral stimulation of carotid bodies with close arterial injection of adenosine causes dose-dependent increase in minute ventilation and blood pressure with a concomitant decrease in heart rate in conscious humans. The ventilatory response was abolished following carotid body ablation but the hemodynamic response partially remained.

- We found that the magnitude of adenosine evoked responses in minute ventilation and blood pressure was analogous to the responses evoked by hypoxia. In contrast, opposing heart rate responses were evoked by adenosine (bradycardia) versus hypoxia (tachycardia).

- Intra-carotid adenosine administration may provide a novel method for perioperative assessment of the effectiveness of transcutaneous carotid body ablation, which has been recently proposed as a novel treatment strategy for various sympathetically mediated diseases.

Word count: 148

ABSTRACT

Stimulation of peripheral chemoreceptors by acute hypoxia causes an increase in minute ventilation (MV), heart rate (HR) and blood pressure (BP). However, the contribution of particular chemosensory areas - carotid (CB) vs. aortic bodies - to this response in humans is unknown. We performed a blinded, randomized and placebo controlled study in 11 conscious patients (9 men, 2 women) undergoing common carotid artery angiography. Doses of adenosine ranging from 4 to 512 µg or placebo solution of matching volume were administered in randomized order via a diagnostic catheter located in a common carotid artery. Separately, ventilatory and hemodynamic responses to systemic hypoxia were also assessed. Direct excitation of a CB with intra-arterial adenosine increased MV, systolic BP, mean BP and decreased HR. No responses in these variables were seen after injections of placebo. The magnitude of the ventilatory and hemodynamic responses depended on both the dose of adenosine used and on the level of chemosensitivity as determined by the ventilatory response to hypoxia. Percutaneous radio-frequency ablation of the CB abolished the adenosine evoked respiratory response and partially depressed the cardiovascular responses in one participant. Our study confirms the contribution of purinergic signaling to chemoreception in humans and suggests that adenosine may be used for selective stimulation and assessment of CB activity. The trial is registered at ClinicalTrials.gov NCT01939912.

Word count: 205

Abbreviations AB - aortic bodies; BP - blood pressure; BR - breathing rate; CA - carotid angiography; CAS - carotid artery stenting; CB - carotid body; DBP - diastolic blood pressure; HR - heart rate; HVR - ventilatory response to hypoxia; IQR - interquartile range; MAP - mean arterial pressure; MV - minute ventilation; R^2 - coefficient of determination; PCh - peripheral chemoreceptors; SBP - systolic blood pressure; SpO_2 - blood oxygen saturation; TIA - transient ischemic attack; TV - tidal volume

INTRODUCTION

Transient hypoxia is known to cause increases in ventilation, blood pressure and heart rate in both human and animal models (O'Regan & Majcherczyk, 1982). This pattern of response is mediated by excitation of peripheral chemoreceptors (PCh), which excites a medullary cardiorespiratory reflex circuit driving autonomic and respiratory motor outputs (Timmers *et al.*, 2003). The primary PCh response to hypoxia consisting of hyperpnoea, bradycardia and a pressor response is modulated by secondary activation of other autonomic reflexes such as the Hering-Breuer reflex, arterial baroreflex and by a direct action of hypoxia on the vasculature (Daly & Scott, 1963; Heistad & Abboud, 1980; Marshall, 1994) and includes hypopnoea, tachycardia and a reduction in vascular resistance.

PCh in humans are located mainly in the carotid (CBs) and aortic bodies (ABs). Although the contribution of the isolated carotid body to the cardiovascular-respiratory response evoked by systemic hypoxia is well described in animals (Daly & Scott, 1958, 1963), not much is known about its function in humans. Previously, we showed that bilateral excision of the CBs, performed as a treatment for congestive heart failure, almost abolished the ventilatory response and reduced the pressor response to systemic hypoxia but the tachycardia persisted (Niewinski *et al.*, 2014a). These data suggest that in humans excitation of various chemosensory areas evoke contrasting primary physiological responses.

Recent studies suggest that overactive PCh play a major role in the pathogenesis of sympathetically mediated diseases. Unilateral or bilateral CB inactivation was proposed as a treatment of human hypertension, heart failure or diabetes (Niewinski *et al.*, 2013b; Paton *et al.*, 2013). While the physiological effect of CB excision in humans with heart failure has been reported, the response to its selective activation in conscious humans with less severe comorbidities is unknown. Evoking such a response would have important clinical implications for patients under consideration for CB modulation therapy as it would: (1) provide a pre-procedural assessment of the functional integrity of either the left or right CB in isolation from the other CB and the ABs (Niewinski, 2014b). (2) indicate whether there is a dominant CB on one side. (3). demonstrate procedural efficacy of unilateral CB ablation post-operatively through an assessment of the magnitude of CB evoked reflex responses.

Recently, it has been reported that experimental blockade of adenosine receptors inhibited CB activity as measured by a reduction in carotid sinus nerve activity in response to hypoxia (Sacramento *et al.*, 2015). Furthermore, adenosine mediated signaling has an excitatory impact on CB sensitivity to hypercapnia (Holmes *et al.*, 2015), thus placing adenosine as a key player in chemotransduction. In terms of an experimental tool for use as a

CB stimulant in humans, adenosine administration is safe and widely used in cardiology. The molecule has an ultra-short plasma half-time (Sollevi, 1986) and is unable to cross the blood-brain barrier (Isakovic *et al.*, 2004). These characteristics make it an ideal stimulatory molecule for transient, selective activation of the CB in humans. Thus, to reveal the ventilatory and hemodynamic effects of unilateral, selective CB stimulation in conscious humans, we performed a single-blinded, randomized study using adenosine injected directly into a common carotid artery.

METHODS

Studied population

After obtaining approval from our local Ethics Committee (Komisja Bioetyczna, Wroclaw Medical University) consecutive patients with significant unilateral internal carotid artery stenosis, referred for carotid artery angiography (CA) or carotid artery stenting (CAS) were invited to enter the study. Subjects excluded included those with significant bilateral lesions of common/internal carotid arteries, impaired left ventricular ejection fraction, suffering from symptomatic pulmonary disease, post stroke/transient ischemic attack (TIA) in last 6 months or acute coronary syndrome in last 3 months and all those presenting contraindications for adenosine administration were excluded. From 107 patients screened the study group consisted of 11 subjects (9 men, 2 women), mean age 66 ($\pm$5) years. Within that group 10 patients had hypertension, 2 had diabetes and 8 had coronary artery disease. All subjects gave an informed consent. The study was performed in accordance with the latest review of the Helsinki Declaration.

Study Protocol

Twenty-four hours preceding the study participants were asked to discontinue all cardiovascular drugs including antihypertensives and β-blockers and to avoid caffeine intake. On the first study day, all subjects underwent standard peripheral chemosensitivity testing using a transient hypoxia method (Chua & Coats, 1995). Unilateral, direct CB stimulation with adenosine was performed during CA/CAS procedure on the next day. All patients were fasted for 6 hours prior to testing.

Measurements

The same procedures and equipment were used during both hypoxic chemosensitivity testing and direct unilateral CB stimulation with adenosine. Subjects were examined in the supine position using a one-way open breathing circuit (Hans Rudolph, Inc., Shawnee, KS, USA). The inspiratory arm of the circuit was connected to a high pressure electric valve, which allowed switching between 100% nitrogen and room air in a silent manner. The expiratory arm was connected via a 1000 L/min flowhead (MLT3000L, ADInstruments) to a differential pressure transducer (FE141 Spirometer, ADInstruments, Sydney, Australia) for the measurement of breathing rate (BR), tidal volume (TV) and minute ventilation (MV). Hemodynamic parameters measured included: heart rate (HR) and blood pressure (BP) that were monitored non-invasively, beat-by-beat using a Nexfin device (BMEYE B.V., Amsterdam, Netherlands). Blood oxygen saturation (SpO_2) was evaluated using a pulse

oximeter (Radical-7, Masimo Corporation Irvine, CA, USA) with an ear clip. All data were collected at a sampling rate of 1 kHz (16-bit resolution) using PowerLab 16/30 (ADInstruments, Dunedin, New Zealand) and recorded on a laptop computer (Dell Inc., Round Rock, TX, USA).

Assessment of individual peripheral chemosensitivity to hypoxia (HVR)

We employed an established method for assessing the sensitivity of PCh to intermittent hypoxia using pulses of 100% nitrogen gas to induce episodes of hypoxia (termed: the hypoxic ventilatory response, HVR; Niewinski *et al.*, 2013a). The HVR was expressed as the slope of the linear regression between the single MV responses and corresponding SpO2 nadirs. Slopes with coefficient of determination (R^2) <0.75 were considered inaccurate and excluded from further analysis.

Assessment of individual hemodynamic response to hypoxia

The HR and SBP responses to acute hypoxia were assessed simultaneously with the HVR. Briefly, the slopes of regression functions were calculated from the peak HR and peak SBP following a hypoxic challenge and corresponding SpO2 nadirs. Linear regression of HR and SBP slopes reflected the magnitude of the hemodynamic responses to acute hypoxia (Niewinski *et al.*, 2013a).

Evaluation of the effects of direct unilateral carotid body stimulation with adenosine

Direct unilateral CB stimulation with adenosine was performed under normoxic conditions during CA/CAS. Before the procedure all subjects underwent carotid ultrasound, which allowed for pre-procedural selection of the investigated side. Adenosine was injected only into non-stenosed common carotid arteries. After carotid arteries angiography, which confirmed lack of significant atheromas (and before stenting, in patients qualified for CAS), the tip of angiographic catheter was positioned 2 cm below the bifurcation of the common carotid artery. For safety reasons, the total duration of the study did not exceed 30 minutes. After 5-minute baseline control period, bolus injections containing various doses of adenosine or placebo (0,9% normal saline solution) were administered via the catheter in a single-blinded order. All injections had the same volume of 5 ml and the same temperature of 36° C. Adenosine boluses (Adenocor, Sanofi-Aventis diluted with 0,9% normal saline solution) were prepared prior to the experiment in sterile conditions and contained the following doses: 4, 8, 16, 32, 64, 128, 256, 384 and 512 µg. After each bolus, subjects were allowed to rest until the measured parameters returned to baseline levels. To determine the effects of bolus injections of adenosine or placebo MV, TV, BR, HR, systolic BP (SBP), diastolic BP (DBP) and mean

arterial pressure (MAP) were averaged from a 80s-period prior to each administration (termed: baseline values). The response to adenosine was defined in two ways as the absolute change in measured parameters between baseline values and: (1) the mean values from a 20s period following the adenosine injection (this is twice the half-life time of adenosine in humans; and (2) the maximal or minimal values from 20s period following the adenosine administration. Additionally, due to the biphasic character of the BP response (Biaggioni *et al.*, 1987), we also analyzed a subsequent 20s epoch for mean changes in measured parameters.

In one subject undergoing unilateral carotid body ablation performed using an investigational catheter system (CIBIEM, Los Altos, CA, USA; NCT02099851) adenosine (in doses of 384 and 512 μg) was administered 5 to 10 minutes before and after the ablation.

Assessment of individual peripheral chemosensitivity to adenosine

In each subject, 3-7 doses of adenosine were administered; the exact number administered depended on individual tolerability and total duration of the responses and recovery. The ventilatory response to adenosine was calculated as the average of the 3 largest consecutive breaths following the adenosine administration. A ventilatory response to adenosine (AVR) was calculated as the slope of the linear regression between the doses of adenosine and the evoked MV responses. Only recordings with at least three successful adenosine administrations and coefficient of determination (R^2) ≥ 0.75 were incorporated into further analysis.

Data and Statistical Analysis

Statistica 12 (StatSoft Inc., Tulsa, USA), LabChart 7 Pro (ADInstruments, Dunedin, New Zealand) and MATLAB (MathWorks, Natick, MA, USA) were used to analyze the data. The distribution of the variables was tested using Shapiro-Wilk's W-test. Data are mean and standard deviation, or median and interquartile range (IQR) where appropriate. The statistical comparisons were evaluated using Wilcoxon Matched Pairs Test for non-normally distributed variables and Student's t-test for normally distributed variables. The correlations were calculated with Spearman rank. *P value* < 0.05 was considered statistically significant.

RESULTS

Ventilatory and hemodynamic responses to hypoxia

Assessment of ventilatory (HVR) and hemodynamic responses to hypoxia was performed in all individuals but one, in which R^2 was less than 0.75, was excluded from the analysis. In the studied group the median HVR was 0.61 l/min/SpO$_2$ [IQR 0.49], median HR response to hypoxia was 0.24 bpm/SpO$_2$ [IQR 0.58] and median SBP response to hypoxia was 1.03 mmHg/SpO$_2$ [IQR 0.76]. Detailed information about the hypoxic responses in individual subjects is shown in Table S1 in Supplementary Appendix.

Effects of intra-common carotid artery adenosine bolus injection

In total, 56 bolus injections containing placebo (n=8) or adenosine (n=48) in fixed doses of: 4, 8, 16, 32, 64, 128, 256 or 512 μg were administered. Six administrations were excluded from the analysis due to artefacts (caused by cough, speaking or Nexfin cuff oscillations) during pre- or post-injection period. A typical response to intra-common carotid artery (i.c.) adenosine bolus-injection is shown in Figure 1.

Figure 2 illustrates the mean values from a 20s post-administration period. Administration of adenosine induced significant increases in MV (+6.3 l/min [IQR 5.6]; p<0.01) relative to the baseline, which was not seen with placebo (+0.2 l/min [IQR 0.5]; p=0.33). Augmented MV was the result of raised TV (+0.4 l [IQR 0.41]; p<0.01) with a, paradoxically, diminished BR (-0.96 breaths/min [±2.2]; p<0.01). Concomitantly, there was a transient decrease in HR after adenosine injection (-2.03 bpm [±2.7]; p<0.01) which was not seen following placebo (+0.64 bpm [±1]; p=0.13). The administration of adenosine also caused an increase in mean (+2.68 mmHg [±6.3]; p=0.01) and systolic (+2.72 mmHg [IQR 7]; p<0.01) BP. Such an effect was not observed after placebo (-0.24 mmHg [±2.8]; p=0.72 and +0.89 mmHg [IQR 4.3]; p=0.78 for MAP and SBP respectively). DBP was influenced neither by adenosine (+1.07 mmHg [±4.2]; p=0.13) nor placebo (-1.55 mmHg [±4.6]; p=0.37).

When peak responses were analysed (Fig. 3.), adenosine administration increased MV (+10.2 l/min [IQR 10.1]; p<0.01), MAP (+7.4 mmHg [IQR 9.2]; p<0.01), SBP (+11.4 mmHg [IQR 9.6]; p<0.01) and decreased HR (-8.3 bpm [±5.2]; p<0.01) compared to baseline. Minimal and maximal values of measured parameters following adenosine injections were significantly different from the placebo evoked responses (Fig 3; P<0.05).

Fig 2 shows the mean values from the time period between 20 and 40 s after adenosine injection. A small but significant fall in MAP (-2.17 mmHg [±6.22]; p=0.04) with

insignificant changes in SBP (-3.69 mmHg [IQR 11.17]; p=0.27) and DBP (-1.09 mmHg [±4.6]; p=0.16) were observed compared to baseline values. HR was slightly lower (-0.78 bpm [±1.81]; p=0.01) but no change in MV (+0.07 l/min [±4.1]; p=0.81) was found compared to baseline.

Latency of onset of the response to adenosine stimulation of a carotid body

The onset of the ventilatory response to adenosine, defined as the time from the bolus injection to the first deep breath (as defined by ≥25% higher TV comparedto the previous breath) was 5.69±1.89s. The onset of the hemodynamic response, defined as the time from the injection to a point at the timeline when the trend to rise or fall in measured parameters appears (as assessed independently by two researchers) was recorded on average at 7.15±2.84s and 4.15s [IQR 1.95] for SBP and HR, respectively.

Dose-dependence of the response to adenosine

Figure 4 shows the magnitude of MV and HR responses correlated linearly with the dose of adenosine used (R=0.7; p<0.01 and R=0.58; p<0.01 for mean and maximal MV, respectively; R=-0.41; p<0.01 and R=-0.41; p<0.01 for mean and minimal HR, respectively). SBP and MAP responses were also dose-dependent. These responses reached statistical significance only for maximal BP values but not for mean BP values (R=0.26; p=0.09 and R=0.39; p=0.01 for mean and maximal SBP respectively, R=0.2; p=0.18 and R=0.33; p=0.03 for mean and maximal MAP respectively).. At all doses the pattern of the respiratory (TV and BR) and haemodynamic (HR and SBP) responses were similar qualitatively.

Individual variability of the response to adenosine and its correlation to baseline variables

The magnitude of the response to particular doses of adenosine differed between patients and depended on the individuals' sensitivity to hypoxia. Generally, the mean increase in MV following adenosine administration was more exaggerated in individuals with higher HVR (R=0.47; p<0.01). Similarly, higher hypoxic SBP response predicted greater mean increase in SBP following adenosine boluses (R=0.34; p=0.04). Interestingly, in subjects with high hypoxic HR response (more exaggerated increase in HR following hypoxic exposure), the mean decrease in HR following adenosine injections was less pronounced - (R=0.39; p=0.02). There was no correlation between HVR and HR / SBP responses to adenosine (R=-0.22; p=0.19 and R=0.11; p=0.5 respectively).

HVR and adenosine induced mean increases in MV correlated with baseline MV (R=0.4; p=0.01 and R=0.7; p<0.01, respectively). There was no relationship between hypoxia/adenosine induced HR and SBP responses and baseline HR and SBP values.

Individual peripheral chemosensitivity to adenosine was calculated in 8 of 11 subjects. In two cases, the assessment was not possible due to a low number of successful adenosine administrations and when R^2 was less than 0.75. Individual peripheral chemosensitivity to adenosine was 0.046 l/min/µg [IQR 0.057] and correlated linearly with HVR (R=0.81; p=0.01). Detailed information about the ventilatory response to adenosine in individual subjects is shown in Table1.

Response to adenosine after carotid body ablation

CB ablation (n=1), abolished the ventilatory response to adenosine. Adenosine injected i.c. prior to the procedure caused an averaged increase in mean MV of +7.5 l/min (+54.6% of baseline ventilation), which was suppressed dramatically after the procedure (+0.72 l/min or +6.3% of baseline ventilation; Fig. 5). Changes in hemodynamic parameters were also diminished following CB ablation (mean change in: MAP +5.71 mmHg vs. +0.37 mmHg; SBP +3.82 mmHg vs. -0.74 mmHg; HR +3.16 bpm vs. +1.3 bpm, for pre- and post-procedural administrations, respectively). However, a relatively low number of adenosine injections were made and periprocedural usage of propofol and fentanyl should be taken into account when hemodynamic data are considered.

Adverse effects of adenosine injections

No serious adverse events were observed with intra-common carotid artery injections of adenosine. One subject with high AVR and HVR reported dyspnoea after a dose of 128 µg (no higher doses were administered), which resolved within 30s. Another patient reported a headache, which was not time-related with the injection of adenosine and abated spontaneously after the end of the procedure; it remains equivocal as to whether this was related to the adenosine injections.

DISCUSSION

We have, for the first time, described the respiratory and cardiovascular responses to selective unilateral CB stimulation in conscious humans. There are three novel findings of the present study. We found that: (1) selective stimulation of CB in conscious humans leads to increase in MV, SBP, MAP and a decrease in HR; (2) the response to adenosine is dose-dependent; (3)

the magnitude of individual sensitivity to adenosine is directly related to the level of sensitivity to hypoxia. Moreover, we show that the respiratory and hemodynamic effect of intra-common carotid arterial injections of adenosine are mediated by the CB as its removal abolishes the responses.

Adenosine as a carotid body stimulant in humans

In animals, adenosine is a neurotransmitter released from type I cells of PCh in response to hypoxia (Prabhakar, 2000). Exogenous adenosine has been shown to stimulate CBs in animals (McQueen & Ribeiro, 1983; Monteiro & Ribeiro, 1987). Biaggioni et al. (1987) and Watt et al. (1987) suggested a similar role for adenosine in PCh physiology in humans . They infused adenosine directly into the aorta and observed various ventilatory and hemodynamic changes, the direction of which were dependent on the location of the tip of the catheter. When the catheter tip was placed in the ascending aorta, proximally to the branches of the aortic arch, adenosine injections caused hyperventilation, but when it was located in the descending aorta, distally to carotid arteries, no change in MV was recorded. Our study confirms the excitatory effect of adenosine on the CB in several ways: first, the effects of CB stimulation were dose-dependent; second, the response occurred a few seconds after the injection, while the onset of the response to intravenous injection is observed after 20-30s (Watt & Routledge, 1985; Biaggioni *et al.*, 1987); third, the individual peripheral chemosensitivity to adenosine correlated with the HVR; finally, the reflex responses to adenosine were abolished following ipsilateral CB ablation in one patient.

In conscious humans, adenosine administered intravenously or into the ascending aorta causes hyperventilation, however the changes in hemodynamics are not clear. Watt and colleagues reported bradycardia when adenosine was administered intravenously and tachycardia with no change in BP during intra-aortic administration (Watt & Routledge, 1985; Watt *et al.*, 1987). On the contrary, Biaggioni at al. (1987) showed increases in HR and BP in both cases . The differences in these cardiovascular responses may result from the direct vasodilatatory (Collis, 1989) and/or direct negative chronotropic effects of adenosine (Belardinelli *et al.*, 1989) when administered systematically or from activation of modulatory mechanism secondary to hyperventilation e.g. the Hering-Breuer reflex (Daly & Scott, 1963). Also, aortic body co-activation should be taken into consideration with systemic administrations, as adenosine was found to increase activity of these PCh in the cat (Runold *et al.*, 1990).

To evaluate the response of the CB selectively we injected low-doses of adenosine directly into the common carotid artery, using the angiographic catheter located 2 cm below

its bifurcation to prevent potential backflow of the chemical into the aortic arch. This, together with ultra-short plasma halftime of adenosine (reported to be less than 10s) (Klabunde, 1983; Sollevi, 1986), suggest that concurrent AB stimulation and direct cardiovascular actions are unlikely. Central nervous effects of adenosine are also unlikely because it does not cross brain-blood barrier (BBB) (Isakovic *et al.*, 2004). It was also shown that enzymes located within endothelial cells that form an enzymatic BBB metabolize nearly 90% of adenosine infused into the internal carotid artery; this would further prohibit potential central nervous system actions of adenosine injected into a common carotid artery (Pardridge *et al.*, 1994). Moreover, at least 50% of drugs administered into the common carotid artery will flow into the external carotid artery and never reach the cerebral circulation.

Selective carotid body stimulation - heart rate response

The cardiovascular response to hypoxia or adenosine depends on an interplay between the: (1) primary response of PCh excitation; (2) secondary modulatory mechanisms (recruited by the primary response) of pulmonary stretch receptors and the arterial baroreflex, which can diminish or even reverse the primary response (Daly & Scott, 1963; Heistad & Abboud, 1980; O'Regan & Majcherczyk, 1982; Marshall, 1994); and (3) direct actions of the stimuli on the vasculature, heart and central nervous system (pressor effect of hypoxia).

To our knowledge there is no published data regarding the effects of selective CB stimulation in humans, however, such an experiment has been performed in animals. Isolated stimulation of CB employing perfusion of carotid arteries with hypoxic blood in anesthetized, artificially ventilated dogs resulted in an increase in BP and a decrease in HR (Daly & Scott, 1958, 1963). Artificial ventilation under conditions of neuromuscular blockade has allowed investigators to disentangle one of the secondary mechanisms – the pulmonary stretch receptor reflex, which decreases vascular resistance (Daly & Scott, 1963) and increases HR via central vagal inhibition in response to increased TV (Paintal, 1973) thereby reducing the primary PCh reflex haemodynamic responses. In spontaneously breathing dogs, hyperventilation was observed, however HR and BP responses varied between animals and most likely depended on the degree of pulmonary stretch receptor activation and thus the magnitude of the hyperventilation (Daly & Scott, 1958, 1963).

In our study in conscious, spontaneously breathing humans, a significant decrease in HR was observed after selective CB stimulation with adenosine. Nevertheless, the magnitude of the HR response may be underestimated due to concurrent activation of the secondary modulatory mechanisms described above. Consistent with opposing effects of the pulmonary stretch receptors on the bradycardia evoked by selective CB stimulation, we observed a lower

nadir HR response that was associated with higher MV responses to adenosine (R=-0.34; p=0.04). This association was for peak TV (R=-0.35; p=0.03), but not for peak BR (R=-0.06; p=0.7) consistent with the increase in TV but not BR during unilateral CB stimulation. The negative chronotropic effect of selective CB stimulation predomined over any positive chronotropic effect evoked from pulmonary stretch receptors; based on their HVR this was most likely assisted by the CB hyperreflexia observed in these patients .

In contrast to the effects of selective CB activation, systemic hypoxia or intravenous adenosine infusion causes increase in HR, which may not be attributed to Heurig-Breuer reflex activation only. As we reported previously, bilateral excision of CBs almost abolished MV and BP responses to hypoxia, however, despite a lack of pulmonary stretch receptor stimulation (secondary to the CB evoked hyperventilation), the increase in HR following hypoxic exposure remained unchanged (Niewinski *et al.*, 2014a). Thus, we suggest that this tachycardic response is related to activation of other primary chemosensory areas e.g. ABs. Moreover, the current study revealed a low magnitude bradycardia to selective CB stimulation in subjects with enhanced hypoxia-induced tachycardia. This observation may be explained by a predominating AB evoked tachycardia during simultaneous CB and AB stimulation with hypoxia. It also suggests the existence of a central interaction between CBs and ABs. The differences in HR response to CB and AB activation might be explained from an evolutionary point of view. In lower vertebrates, chemoreceptors located mainly on the first gill arch (which evolve to form mammalian CBs) are responsible for sensing environmental oxygen partial pressure rather than blood oxygen levels. Responses mediated by these receptors serves to minimize the effects of environmental hypoxia by hyperventilation (increased oxygen uptake) and by bradycardia (improved oxygen-conservation). On the other hand, arterial chemoreceptors (located on other gill arches), which evolve to form the ABs, monitor blood oxygen content (SpO_2) and respond functionally to ensure adequate oxygen delivery to the tissues, which may explainthe tachycardia following their stimulation (Milsom & Burleson, 2007).

Selective carotid body stimulation - blood pressure response

The analysis of BP curves during bolus injections of adenosine revealed a significant increase in SBP and MAP during the first 20s and a decrease in MAP compared to baseline levels during the subsequent 20s. This biphasic BP response was commonly observed in our previous studies during HVR testing (Niewinski P, Tubek S - unpublished data) and by Biaggioni et al. (1987) after intravenous adenosine bolus-injections . The first phase of the BP response is associated with the primary PCh evoked sympathoexcitation (Lugliani *et al.*,

1973; Guyenet, 2000; Niewinski *et al.*, 2014a), the genesis of the second phase is unclear. We hypothesize that this phase may be attributed to activation of arterial baroreceptors. This hypothesis is further supported by a decrease in HR that is observed during second phase of MAP response.

Selective carotid body stimulation - minute ventilation response

Stimulation of PCh leads to hyperventilation, which in humans is mainly dependent on the increase in TV, rather than BR (Caruana-Montaldo *et al.*, 2000). The contribution of particular chemosensory areas to the magnitude of the ventilatory response in mammals is estimated to be 90% for CBs and 10% for ABs (Caruana-Montaldo *et al.*, 2000); this ratio was also found in in our previous humans studies (Niewinski *et al.*, 2014a). Whether there is any difference between CBs and ABs in the pattern of the response (rise in TV vs. BR) is not known. However, a recent study suggested that CBs are mostly drive an increase in TV whereas ABs increase BR (Smith & Mills, 1980)..

Among previous studies in humans employing adenosine (given intravenously) as a stimulant of PCh, variability in the pattern of the ventilatory response was found. Biaggioni et al. (1987) reported an increase in TV with no change in BR following administration of adenosine i.v., while others observed an increase in both TV and BR (Watt & Routledge, 1985). This difference, may reflect contribution from the ABs (increasing BR) and CBs responsible for increase in TV. According to our results, selective CB stimulation led to hyperventilation caused by an increase in TV with paradoxically a decrease in BR. This suggests that in humans CBs are predominantly responsible for regulation of TV but we cannot rule out that the pattern of response may change in humans with PCh hyperreflexia and cardiovascular disease.

The magnitude of the ventilatory response to selective carotid body stimulation expressed as individual ventilatory response to adenosine depends on the HVR assessed with our transient hypoxia method. Our data confirms the role of adenosine in chemotransduction in conscious humans. Further, we propose that intra-common carotid artery injections of adenosine may be used as a test for the assessment of CB function such as before and after CB ablation as a procedural efficacy test; however this method needs further validation.

Endovascular carotid body ablation

The ventilatory response to intra-common carotid artery injection of adenosine was lower in the subject that under went subsequent CB ablation compared to other subjects. This

difference may be explained by the need to use anaesthetic medications (fentanyl and propofol) during the procedure. Both drugs are known to blunt ventilatory and hemodynamic responses to CB stimulation (Weil *et al.*, 1975; Mayer *et al.*, 1989; Jonsson *et al.*, 2005). However, the changes in MV and BP responses to adenosine following the ablation were evident despite sedation. Similarly, the lack of a bradycardia in the pre-procedural assessment may be attributed to drug-related inhibition of parasympathetic cardiac tone (Sato *et al.*, 2005) and the tachycardia, secondary to hyperpnoea, becoming dominant. .

Study limitations

Despite selective stimulation of CBs, we were unable to deactivate all secondary modulatory mechanisms recruited by the primary response such as reflexes mediated by pulmonary stretch receptors and baroreceptors. Thus, the magnitude of changes in recorded parameters may be underestimated. Also, there is some data suggesting vasodilatatory influence of systematically infused adenosine on brain vasculature (Sollevi *et al.*, 1987), which may lead to increased CO_2 wash out and a rise in local pH and a desensitization of central chemoreceptors. However, such a side effect of adenosine, if it exists, would not affect the initial response. Finally, we did not record sympathetic activity, which would provide a more comprehensive explanation for the observed hemodynamic changes.

CONCLUSION

In the present study, we present novel insights into the physiology of selective unilateral CB stimulation in conscious humans. We found that bolus administration of adenosine given in close proximity to a CB leads to a decrease in HR, which is different from systemic activation of PCh (e.g. when hypoxia is used) most likely due to an elimination of the concurrent stimulation of Abs and secondary recruitment of pulmonary stretch receptors. Moreover, we noted that the ventilatory response to hypoxia is closely related to the response evoked by adenosine injection into the common carotid artery, which further confirms a functional role of adenosinergic signalling in physiological chemotransduction in humans. Adenosine given into the common carotid artery constitutes a novel approach for the study of the physiology and sensitivity of an isolated CB.

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Additional information

Conflict of interest

Funding

Author contributions

The experiments were performed in Laboratory for Applied Cardiovascular Research at Department of Cardiology, 4th Military Hospital, Wroclaw, Poland.

All authors approved the final version of the manuscript. All persons designated as authors qualify for authorship. All those who qualify for authorship are listed.

Acknowledgements

None

Fig. 1. Typical ventilatory and hemodynamic responses to intracarotid adenosine injection.
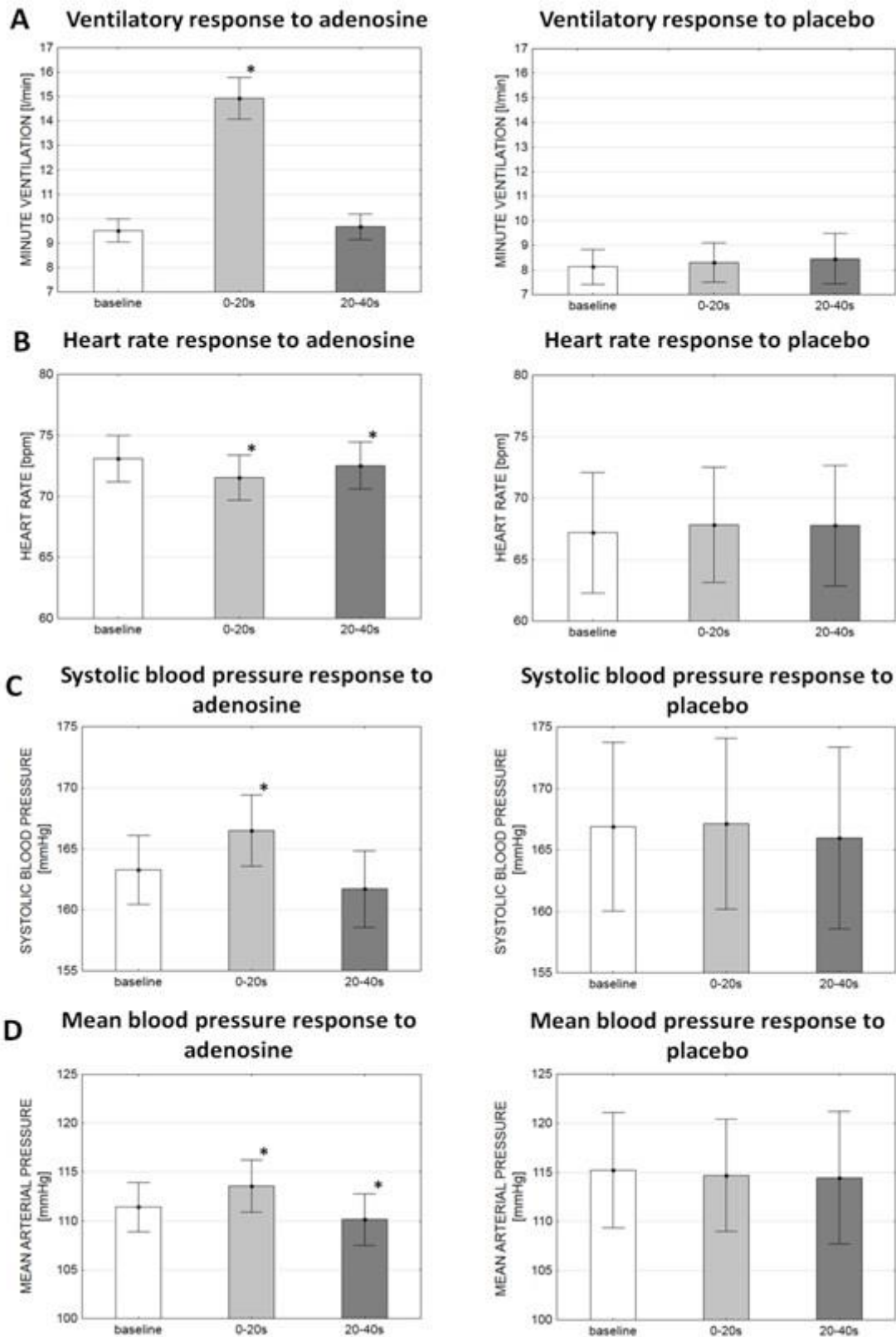
Fig. 2. Mean ventilatory and hemodynamic responses to intracarotid adenosine injections. Open columns - mean baseline parameters, light-gray columns - mean values from 20s period following the administration of adenosine, dark-gray columns - mean values from the time period between 20 and 40 second after adenosine injection. Data are presented as mean ± standard error of the mean. *p<0.05 vs. baseline.
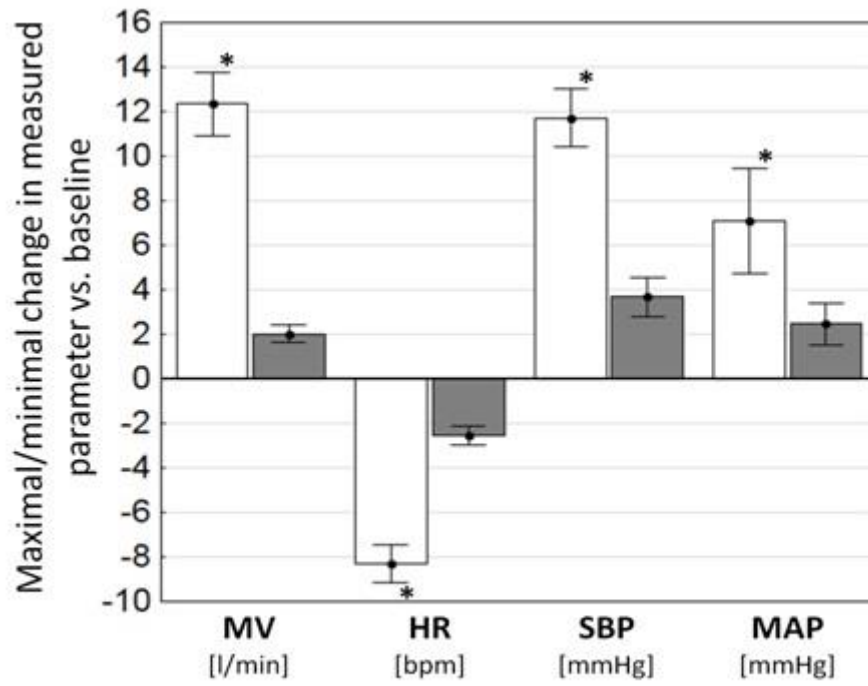
Fig. 3. Comparison of the peak absolute changes between baseline recording and maximal / minimal values from 20 s period following adenosine (open columns) and placebo (shaded columns) injections. Data are presented as mean ± standard error of the mean. *$p<0.05$ vs. placebo.
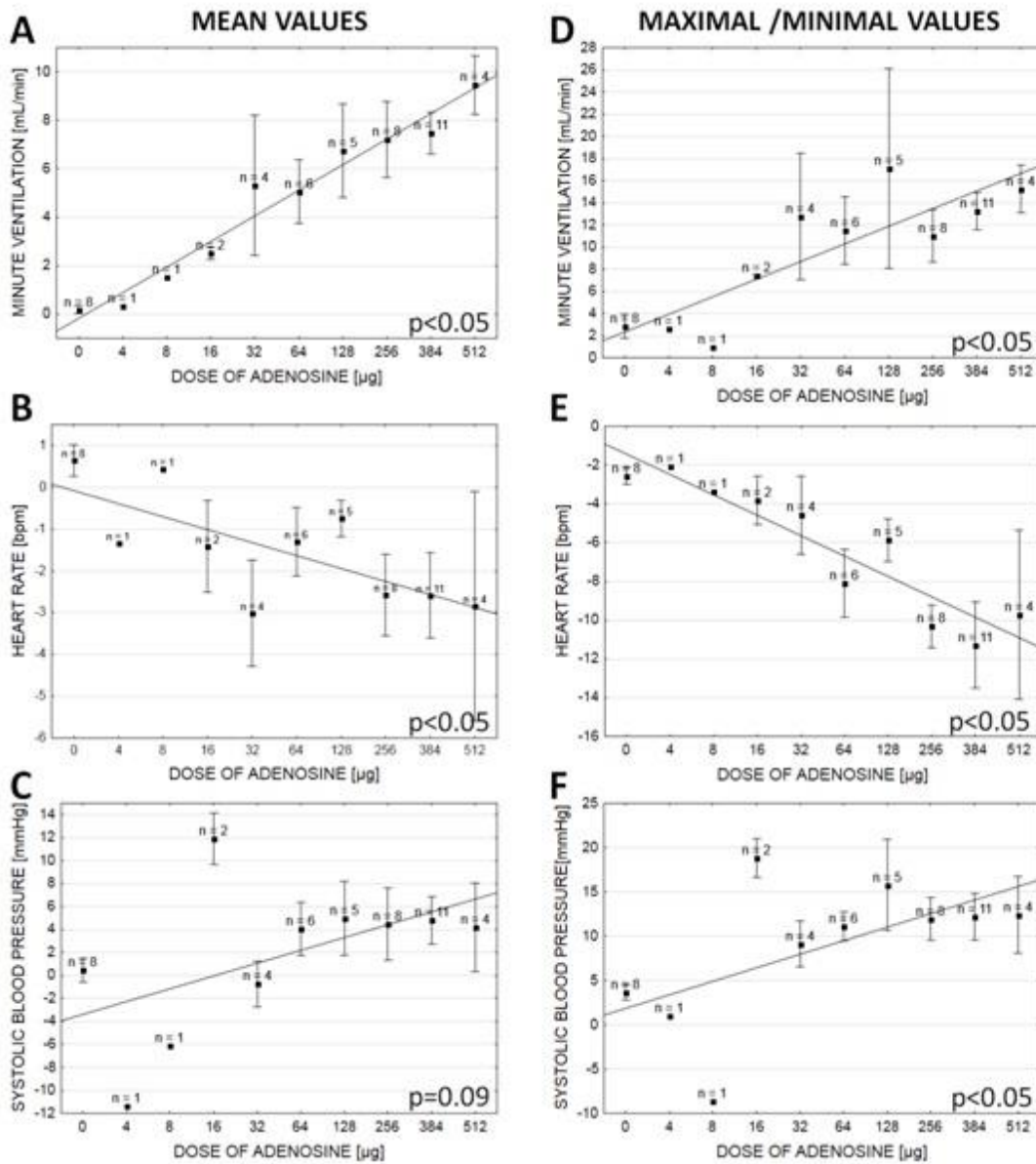
Fig. 4. Dose dependence of the ventilatory and hemodynamic responses evoked by adenosine injections injected into a common carotid artery. Left column displays changes in measured parameters between baseline recordings and mean values from 20s period following adenosine injection in relation to the adenosine doses (A-C). Right column shows changes in measured parameters between baseline recordings and maximal / minimal values from 20s period following adenosine injection in relation to the adenosine doses (D-F). Data are presented as mean ± standard error of the mean.
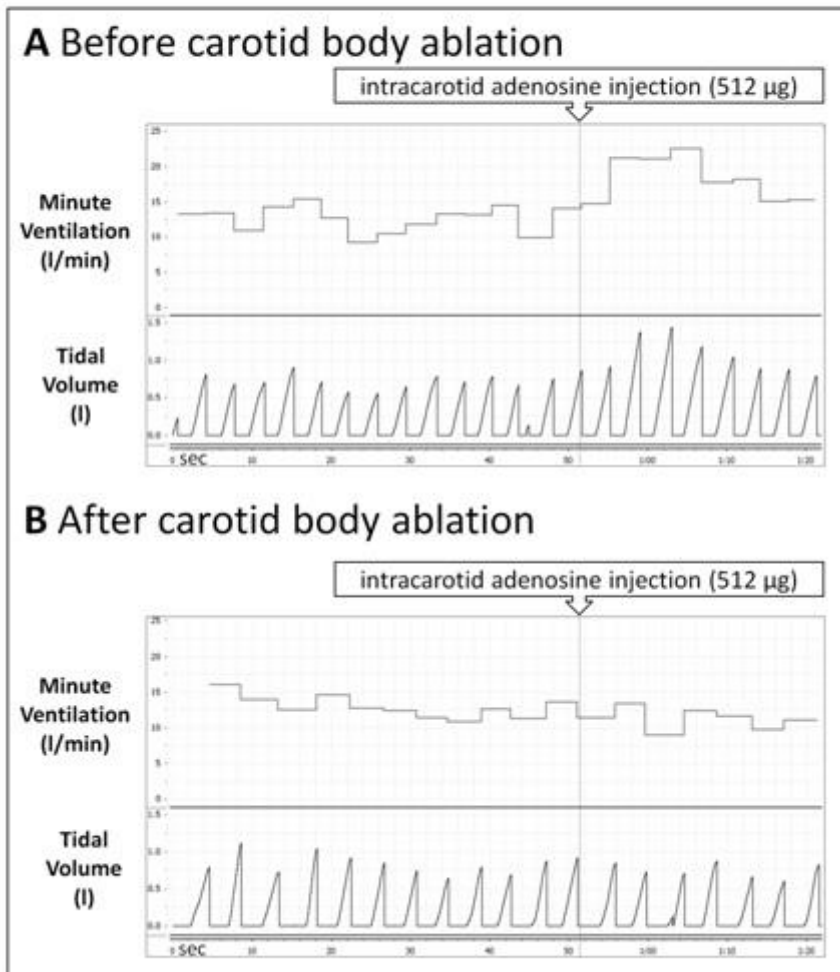
Fig. 5. Ventilatory response to intracarotid injection of adenosine before (A) and after (B) carotid body ablation in the same patient.

Supplementary Appendix

Tab. S1. Individual responses to hypoxia and to intra-carotid adenosine administration.

Subject No.	peripheral chemosensitivity to hypoxia (HVR) l/min/SpO$_2$	heart rate response to hypoxia bpm/SpO$_2$	systolic blood pressure response to hypoxia mmHg/SpO$_2$	peripheral chemosensitivity to adenosine l/min/μg
01	0.470	0.170	1.699	0.021
02	0.312	0.038	0.779	-†
03	0.760	1.781	0.663	0.045
04	0.572	0.620	0.397	0.029
05	0.655	0.171	0.879	0.107
06	2.329	0.317	1.502	0.276
07	-*	-*	-*	-†
08	0.478	0.480	0.641	0.030
09	0.943	0.712	2.302	0.067
10	0.963	0.007	1.182	0.047
11	1.249	0.736	1.537	-*

* not calculated due to low R^2 (<0.75)

† not calculated due to low number of successful administrations of adenosine