11 Adenosine Mediates Hypercapnic Response in the Rat Carotid Body via A_{2A} and A_{2B} Receptors

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Abstract

Adenosine is one of the key neurotransmitters involved in hypoxic signaling in the carotid body (CB), and it was recently found to have a modulatory role in mediating hypercapnic sensitivity in the CB. Herein we have investigated the contribution of adenosine to the hypercapnic response in the rat CB and studied the adenosine receptors responsible for this effect. Experiments were performed in *Wistar* rats. Adenosine release in normoxia (21% O_2) and in response to hypercapnia (10% CO_2) was quantified by HPLC. Carotid sinus nerve (CSN) chemosensory activity was evaluated in response to hypercapnia in the absence and presence of ZM241385 (300 nM), an A_2 antagonist, and SCH58261 (20 nM), a selective A_{2A} antagonist. Hypercapnia increased the extracellular concentrations of adenosine by 50.01%. Both, ZM241385 and SCH58261, did not modify significantly the basal frequency of discharges of the CSN. Also, ZM241385 and SCH58261 did not modify the latency time and the time to peak in CSN chemosensory activity. CSN activity evoked by hypercapnia decreased by 58.82 and 33.59% in response to ZM241385 and to SCH58261, respectively. In conclusion, the effect of adenosine in mediating the hypercapnic response in the rat CB involves an effect on A_{2A} and A_{2B} adenosine receptors.

Keywords

Adenosine · Adenosine receptors · Carotid body · Carotid sinus nerve activity · Hypercapnia

11.1 Introduction

The carotid body (CB) is an arterial chemoreceptor activated by decreases in blood PaO_2 and pH or increases in blood $PaCO_2$ (Gonzalez et al. 1994). The CB plays a homeostatic role in response to high $PaCO_2$ and low pH and, it seems that approximately to 30–50% of the respiratory drive produced by arterial hypercapnia is mediated by CB (Heeringa et al. 1979; Rodman et al. 2001), with the remaining contribution arising from central chemoreceptors (Nattie 1999). Also, CB afferent activity is essential to set hypercapnic sensitivity in central chemoreceptors (Blain et al. 2010; Smith et al. 2015). While the transduction mechanisms and the neurotransmitters involved in the CB response to hypoxia are well studied, the CB chemotransduction in response to hypercapnia has received less attention. In 2015, Holmes and collaborators have

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demonstrated that adenosine have a modulatory role in mediating hypercapnic sensitivity in the CB. Herein, we have investigated the role of adenosine, a key excitatory neurotransmitter involved in hypoxic signalling in the CB (Conde et al. 2009, 2012), on the CB response to hypercapnia. The role of adenosine was studied by testing ZM241385, an A_2 antagonist, and SCH5826, a selective A_{2A} antagonist on basal CSN chemosensory activity and in response to hypercapnia. We also evaluated the CB release of adenosine in response to hypercapnia. We found that hypercapnia induced the CB release of adenosine and that this mediator is involved in the hypercapnic response in the rat CB via both A_{2A} and A_{2B} adenosine receptors.

11.2 Methods

Experiments were performed in *Wistar* adult rats (300–380 g) obtained from *vivarium* of the NOVA Medical School|Faculdade de Ciências Médicas (NMS|UNL), Universidade Nova de Lisboa. Animals were kept under temperature and humidity control (21 ± 1 °C; 55 ± 10% humidity) with a 12 h light/12 h dark cycle. Animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.), tracheostomized and the carotid arteries were dissected past the carotid bifurcation. To quantify adenosine at the CB, the CBs were cleaned free of CSN nearby connective tissues under dissection microscope and incubated in Tyrode solution (Conde et al. 2012). For CSN recordings, the preparation CB-CSN was identified and processed for CSN recordings as previously described by Rigual et al. (2002) and Conde et al. (2012). In all instances animals were killed by intracardiac overdoses of sodium pentobarbital. Principles of laboratory care were followed in accordance with the European Union Directive for Protection of Vertebrates Used for Experimental and Other Scientific Ends (2010/63/EU). Experimental protocols were approved by the ethics committee of the NMS|UNL.

11.2.1 CB Adenosine Release in Response to Hypercapnia

CBs were cleaned free of CSN nearby connective tissues under dissection microscope and incubated in Tyrode solution (Conde et al. 2012). To evaluate the release of adenosine from CBs in response to hypercapnia (10% CO_2) in control animals, the CBs were incubated in 500 mL of Tyrode bicarbonate solution with 2.5 μM of EHNA (an inhibitor of adenosine deaminase) and 5 μM of S-(p-nitrobenzyl)-6-thioinosine (NBTI, an inhibitor of equilibrative nucleoside transport system). Solutions were kept at 37 °C and continuously bubbled with normoxia ($20\%O_2$ + $5\%CO_2$ + balanced N_2), except when hypercapnic stimuli was applied ($20\%O_2$ + $10\%CO_2$ + balanced N_2). Protocol for adenosine release included: 2 periods of 10 min normoxic incubation, followed by 10 min incubation in hypercapnia and 2 post-hypercapnia incubations in normoxia during 10 min. The solutions were renewed at each fixed time, and all fractions were collected and quantified by HPLC as previously described (Conde et al. 2012). At the end of the experiment, the CBs have been placed in PCA 3 M and processed for the quantification of adenosine content.

11.2.2 CSN Electrophysiological Recordings

Extracellular recording from single or few fibers of CSN were performed using a suction electrode. The pipette potential was amplified (Neurolog Digimiter, Hertfordshire, UK), filtered with low pass (5 kHz) and high pass (10 Hz) filters, digitized at 5 kHz (Axonscope, Axon Instruments, Molecular Devices, Workingham, UK) and stored on a computer. Chemoreceptor activity was identified (spontaneous generation of action potentials at irregular intervals) and confirmed by its increase in response to hypoxia (0%O2 + 5% CO_2 + balanced N_2), which corresponds approximately to 20 mmHg, (Conde et al. 2012).

CSN unit activity was converted to logic pulses, which were summed every second and converted in a voltage proportional to the sum. The effect of ZM241385 (300 nM), an A_2 antagonist, and SCH58261 (20 nM), a selective A_{2A} antagonist, on the CSN activity has been investigated while perfusing the preparations with normoxic (20% O_2-equilibrated) and hypercapnic (10% CO_2-equilibrated) solutions.

11.2.3 Statistical Analysis

Data were evaluated using GraphPad Prism Software, version 6 and was presented as the mean ± SEM. The significance of the differences between the means was calculated by unpaired t test and one-way analysis of variance (ANOVA) with Bonferoni's multiple comparison tests. Differences were considered significant at $p < 0.05$.

11.3 Results

Figure 11.1 shows the effect of hypercapnia on adenosine release from the rat CB. Hyperca-pnia (10% CO_2) increased significantly the extracellular concentrations of adenosine with the release reaching 105.70 ± 11.45 (pmol/mg tissue), when compared with the basal normoxic release (before hypercapnia = 70.42 ± 8.83 pmol/mg tissue; after hypercapnia = 82.89 ± 22.13 pmol/mg tissue).

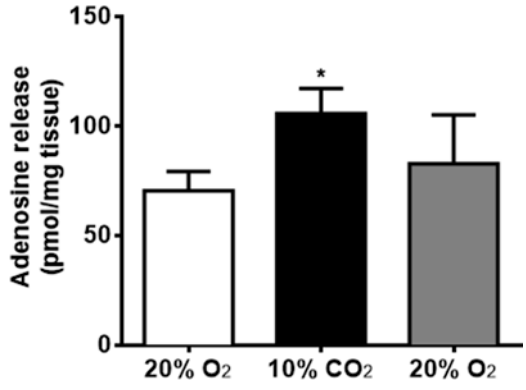

Fig. 11.1 Effect of hypercapnia (10% CO_2) on adenosine release from the rat CB. Protocol included 2 periods of 10 min in normoxia (20%O_2 + 5%CO_2), followed by 10 min of hypercapnia (20%O_2 + 10% CO_2) followed by two normoxic period (n = 6). Data are means ± SEM. Unpaired t test, *$p < 0.05$

Figure 11.2 depicts the effect of ZM241385 (300 nM), an A_2 antagonist, and SCH58261 (20 nM), a selective A_{2A} antagonist, on the CSN chemosensory activity in response to hypercapnia. Basal CSN chemosensory activity was not modified by the application of both adenosine receptors antagonists (Fig. 11.2a). The application of ZM241385 and SCH58261 showed a non-significant tendency to increase the onset of the response (latency time) (Fig. 11.2b). However, none of the drugs tested modified significantly the time to reach the maximal activity (time to peak, Fig. 11.2c).

Figure 11.2d represents typical recordings of CSN chemosensory activity in response to hypercapnia in control conditions, in the absence of any drugs, and in the presence of ZM241385 and SCH58261. The application of both drugs decreased the CSN activity in response to hypercapnia. However, ZM241385 was more effective in inhibiting CSN activity than SCH58261 (Fig. 11.2d). This result was confirmed when we plot the area under the curve (AUC) for the CSN chemosensory activity elicited by hypercapnia in control conditions and in the presence of ZM241385 and SCH58261 (Fig. 11.2e). ZM241385 decreased the AUC by 58.82% when compared with the effect of hypercapnia in the absence of the drugs, while SCH58261 decreased the AUC by 33.59% (Fig. 11.2e; AUC control = 2150.0 ± 194.2; AUC ZM241385 = 885.2 ± 153.2; AUC SCH58261 = 1428 ± 93.2 spikes/s).

11.4 Discussion

The present study demonstrates that adenosine mediates the CSN chemosensory activity evoked by hypercapnia, an effect that is mediated by A_2 adenosine receptors. We have showed that both adenosine receptor antagonists tested did not modify basal CSN chemosensory activity, the latency time and the time to peak. Additionally, both ZM241385 and SCH58261 decreased the

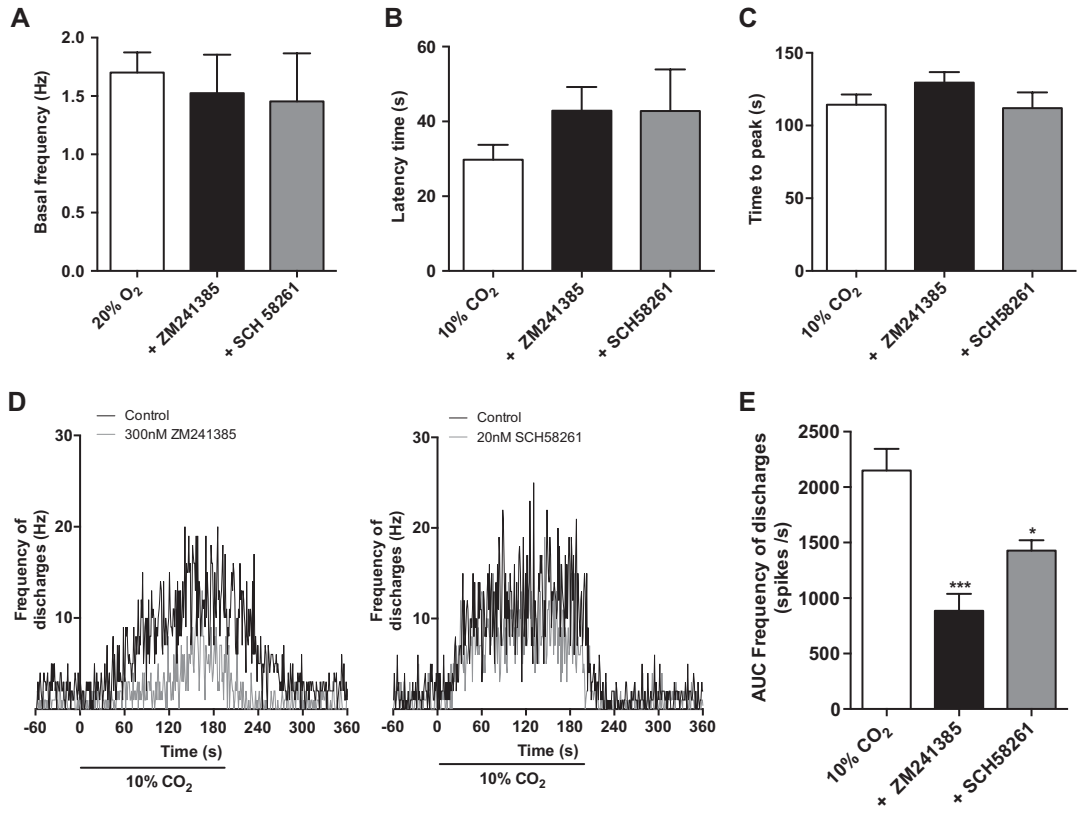

Fig. 11.2 Effect of ZM241385 (300 nM), an A_2 adenosine receptor antagonist, and SCH58261 (20 nM), a selective A_{2A} antagonist, on carotid sinus nerve (CSN) chemosensory activity elicited by hypercapnia (10%CO_2). (**a**) Mean basal frequencies. (**b**) Latency time (the onset of the response). (**c**) Time required to reach maximal activity (time to peak). (**d**) Typical recordings of the effect of ZM241385 and SCH58261 on the frequency of action potentials of CSN during superfusion with a solution equilibrated with 10%CO_2, respectively. (**e**) Area under the curve (AUC) obtained from the analysis of the curves plotted in D. Data represent means ± SEM of 5–7 animals. One-way ANOVA with Bonferroni multiple comparison tests, $^*p < 0.05$ and $^{***}p < 0.001$ *vs* control values

hypercapnia-evoked CSN chemosensory activity, being the effect more pronounced when ZM241385 was applied, suggesting that apart from A_{2A} receptors, A_{2B} adenosine receptors also contribute to this effect.

We have observed that hypercapnia induced the release of adenosine from the CB, as it happens in response to hypoxia (Conde and Monteiro 2004). In the present study we did not evaluate the source of the extracellular adenosine, however we can postulate the main origin of extracellular adenosine induced by hypercapnia could be the extracellular ATP catabolism, since Holmes et al. (2015) showed that the application of an 5′-ectonucleotidase (CD73) inhibitor, α,β-methylene ADP (AOPCP), dramatically (98%) decreased the CB sensitivity to hypercapnia.

In accordance with our previous findings we have observed herein that ZM241385 and SCH58261 did not modify the basal CSN activity (Conde et al. 2012), which suggest that adenosine did not contribute significantly to fix the basal activity of the CB in control conditions. Moreover, the application of ZM231485 and SCH58261 decreased the CSN activity elicited by hypercapnia, meaning that the effect of hypercapnia in the CB is mediated by A_2 adenosine receptors. Additionally, the fact that ZM241385, the non-selective A_2 antagonist decreased more the response to hypercapnia than SCH58261, the selective A_{2A} antagonist, indicated that both A_{2A} and A_{2B} adenosine receptors contribute to the effect of hypercapnia on the CB. In fact, from the observation of the Fig. 11.2d, A_{2A} seems to con-

tribute with approximately 30% for the CSN activity in response to hypercapnia and A_{2B} with approximately 25%. These values are quite similar with those observed for the contribution of adenosine A_{2A} and A_{2B} receptors for the responses to moderate hypoxia in the CB (Conde et al. 2006). However, since we have not tested more intense hypercapnic stimuli we cannot anticipate if these contributions are maintained with higher or lower hypercapnic intensities. Both A_{2A} and A_{2B} adenosine receptors are Gs-coupled receptors and therefore adenosine action through these receptors involves the increase in cAMP levels (Sebastião and Ribeiro 2009). Herein, we did not studied the modifications in cAMP levels induced by hypercapnia, but Holmes and co-workers (2015) have showed that SQ2236, an inhibitor of transmembrane adenylyl cyclases, decreased the elevation evoked by hypercapnia by approximately 50%, a value that is quite similar with the 58% of reduction in CSN activity achieved by us with the non-selective blocker of A_2 receptors in hypercapnia.

In conclusion, the effect of adenosine in mediating the hypercapnic response in the CB is mediated by both A_{2A} and A_{2B} adenosine receptors.

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