5 Molecular Characterization of Equilibrative Nucleoside Transporters in the Rat Carotid Body and Their Regulation by Chronic Hypoxia

Shaima Salman and Colin A. Nurse

Abstract

The mammalian carotid body (CB) is the main peripheral arterial chemoreceptor organ that is excited by decreases in blood PO_2 (hypoxia) and increases in blood PCO_2/H^+. An increase in CB afferent carotid sinus nerve (CSN) discharge results in respiratory and cardiovascular reflex responses that help maintain homeostasis. The CB consists mainly of innervated clusters of the chemoreceptive type I (glomus) cells that are associated with the processes of glial-like type II cells. Extracellular ATP and adenosine (ADO) levels increase in response to acute hypoxia and there is evidence that during chronic sustained hypoxia ADO elevation plays a major role in regulating CB chemosensitivity and CSN discharge. We recently characterized the molecular identities of ectonucleotidase enzymes involved in regulating extracellular ATP hydrolysis to produce ADO in the rat CB. In the present study, we focus on a molecular characterization of the equilibrative nucleoside transporter (ENT) system that is known to regulate extracellular ADO concentrations in the rat CB based on pharmacological studies. Examination of ENT expression using *quantitative* PCR (qPCR) analysis revealed the expression of both ENT1 and ENT2 mRNAs in whole CB extracts from ~2-week-old juvenile rats. In dissociated rat CB cultures, both ENT1 and ENT2 immunoreactivity was localized to type I cell clusters. Furthermore, we show that ENT1 and ENT2 mRNA expression is downregulated in CBs isolated from rat pups exposed to chronic hypobaric hypoxia (~1 week). These findings reveal the molecular identities of the ENT system expressed in the rat CB and are consistent with the proposed shift to ADO signaling during chronic hypoxia.

Keywords

Equilibrative nucleoside transporter · Adenosine · Carotid body · Hypoxia

5.1 Introduction

Mammalian carotid bodies are the main peripheral arterial chemoreceptors that sense changes in arterial PO_2 and PCO_2/H^+ and elicit respiratory and cardiovascular reflexes to maintain homeostasis (Gonzalez et al. 1994; Kumar and Prabhakar 2012). The functional unit of the carotid body (CB) consists of innervated clusters of chemosensitive type I (glomus) cells that are ensheathed by the elongated processes of type II (glial-like) cells. The type I cells sense a decrease in blood O_2 (e.g. hypoxia) and elevated CO_2/H^+ (acid

S. Salman (✉) · C. A. Nurse
Department of Biology, McMaster University, Hamilton, ON, Canada
e-mail: ssalman7@jhmi.edu

E. B. Gauda et al. (eds.), *Arterial Chemoreceptors*, Advances in Experimental Medicine and Biology 1071, https://doi.org/10.1007/978-3-319-91137-3_5

hypercapnia) and elicit the release of neurochemicals that shape the carotid sinus nerve (CSN) discharge (Kumar and Prabhakar 2012; Lopez-Barneo et al. 2008; Nurse 2010; Nurse and Piskuric 2013; Peers and Buckler 1995).

ATP and adenosine, acting postsynaptically and/or presynaptically via purinergic receptors, play a major role in shaping the CSN discharge in response to chemostimuli (Bairam et al. 2013; Conde et al. 2012a; Kumar and Prabhakar 2012; Nurse 2014; Nurse and Piskuric 2013). Two sources of ATP release have been identified within the CB in response to hypoxia and hypercapnia. ATP released from type I cells acts postsynaptically on P2X2/3 receptors expressed on the CSN terminals (Prasad et al. 2001; Rong et al. 2003; Zhang et al. 2000), or on P2Y2 receptors expressed on glial-like type II cells (Tse et al. 2012; Xu et al. 2003; Zhang et al. 2012), inducing further ATP release through pannexin-1 (Panx-1) channels (Zhang et al. 2012). Furthermore, adenosine, produced via the hydrolysis of ATP by ectonucleotidases or released through the bidirectional equilibrative nucleoside transporters, may act on postsynaptic and presynaptic P1 receptors (Conde et al. 2009, 2012a; Holmes et al. 2017; Murali and Nurse 2016).

Extracellular adenosine may be produced by two major metabolic sources, i.e. hydrolysis of extracellular ATP by the combined action of ectonucleotidases and ecto-5′ nucleotidase, and/or release from type I cells via the Na^+ independent equilibrative nucleoside transport (ENT) system. Both sources have been described in the CB using pharmacological tools, with the latter being responsible for ~45% of the adenosine release during mild hypoxia (Conde et al. 2012b).

We recently provided molecular and immunohistochemical evidence for the presence of surface-located ectonucleotidases NTPDase1, 2 and 3, and ecto-5′ nucleotidase (E5′Nt) in the rat CB (Salman et al. 2017), responsible for the hydrolysis of tri-, di-, and monophosphate molecules to produce adenosine. However, though previous studies demonstrated pharmacological evidence for the presence of the equilibrative transporter system in the rat CB (Conde and Monteiro 2004), information on the molecular identity of the corresponding transporters is lacking. The equilibrative transporter family consists of three subtypes, equilibrative nucleoside transporter (ENT)-1-3 (Baldwin et al. 2004). ENT1 and ENT2 are trafficked to the plasma membrane while ENT3 is trafficked intracellularly to the mitochondria (Baldwin et al. 2005; Govindarajan et al. 2009). In this study, we report on the molecular characterization of ENT1 and ENT2 mRNAs using quantitative PCR, as well as protein localization using immunofluorescence labeling in the rat CB. We show that the rat CB expresses ENT1 and ENT2 mRNA and protein localized to type I cell clusters. In addition, we examined the regulation of ENT1 and ENT2 in CBs obtained from rat pups exposed to chronic hypobaric hypoxia for ~1 week.

5.2 Methods

See Salman et al. (2017). Mouse monoclonal human ENT1 (sc-377283) and ENT2 (sc-373871) antibodies were purchased from Santa Cruz.

5.3 Results

Quantitative real-time PCR (qPCR) analyses of ENT1 and ENT2 mRNA expression in the carotid body relative to brain and peripheral ganglia. Expression of the adenosine transporters, equilibrative nucleoside transported 1 (ENT1) and (ENT2) was determined in extracts of the whole rat carotid body (CB) and compared to the expression pattern in tissues/cells from the central and peripheral nervous system. Products of RT-PCR analysis confirmed mRNA expression of both ENT1 and ENT2 in the rat carotid body. Whole brain tissue extract was used as a positive control for ENT expression in central nervous system (Che et al. 1995). Whole tissue extracts from superior cervical ganglion (SCG) and petrosal ganglion (PG) were used as positive controls for ENT expression in peripheral nervous system. Expression of ENT1 and ENT2 mRNA was detected in the CB, SCG and PG. We also examined ENT expression in an immortalized adrenal

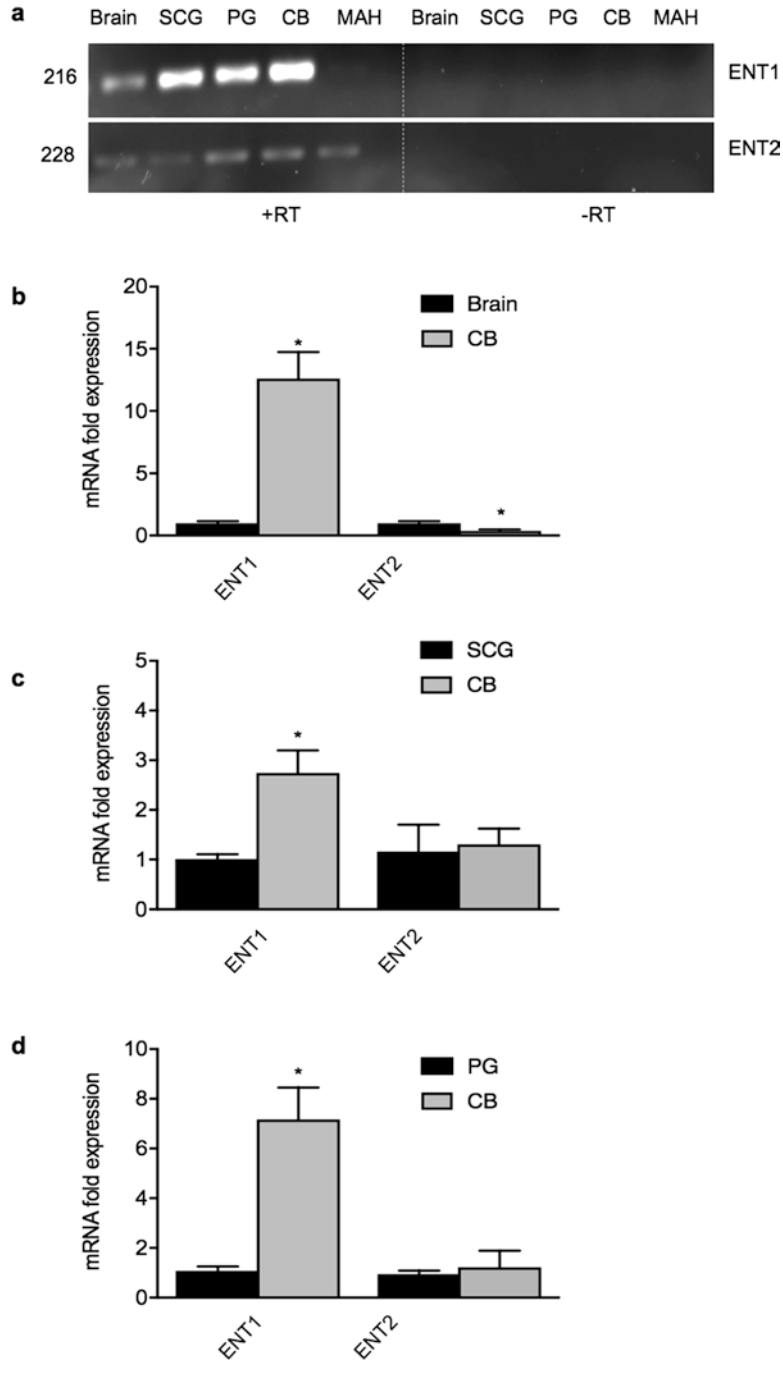

Fig. 5.1 Expression analysis of equilibrative nucleoside transporter 1 (ENT1) and 2 (ENT2) genes in rat carotid body (CB) and tissues/cells from central and peripheral nervous systems. Gel electrophoresis of RT-PCR products for ENT1 and 2 mRNA in rat brain, superior cervical ganglion (SCG), petrosal ganglion (PG), whole CB tissue, and the immortalized chromaffin cell line (MAH). No-Reverse Transcriptase (-RT) samples were used as a negative control (**a**, $n = 3$). *Quantitative* real-time PCR analysis of ENT1 and ENT2 mRNA expression in whole rat CB relative to brain (**b**), or superior cervical ganglion (SCG) (**c**), or petrosal ganglion (PG) (**d**). Experimental replicate represents expression in tissues pooled from individual rat litters (n). Data are presented as means ±SEM of ENT expression in CB tissue relative to specified groups; $n = 4$. Asterisks denote $P < 0.05$ vs. control tissue

chromaffin (MAH) cell line (see Salman et al. 2017). The expression pattern of ENT1 and ENT2 transcripts appeared quite variable among these tissues/cells as shown in Fig. 5.1a. Note the CB expresses transcripts of both ENT1 and ENT2. In contrast to chromaffin-derived PC12 cells which are known to express both ENT1 and ENT2 (Kobayashi et al. 2000), the MAH cell line appeared to express ENT1 at a lower level than ENT2 (Fig. 5.1a; $n = 3$).

When compared with ENT mRNA expression in the brain and SCG, which express adenosine

A1 receptors (Che et al. 1995; Connolly et al. 1993), we found the rat CB expresses significantly higher levels of ENT1 mRNA (Fig. 5.1b, c). Petrosal ganglia (PG), which relay chemosensory information from the CB to the central pattern generator in brainstem are known to express adenosine A_{2a}, A_{2b}, and A_1 receptors (Conde et al. 2012a; Gauda 2000; Nurse 2014; Nurse and Piskuric 2013). As shown in Fig. 5.1d, expression of ENT1 mRNA was significantly higher in the CB relative to the PG. By comparison, expression of ENT2 mRNA did not seem to be differentially expressed in the CB relative to SCG and PG, though there was an apparent lower expression in the CB relative to the brain (Fig. 5.1b). These data suggest that ENT1 is the dominantly expressed member of the nucleoside transporter family in the rat CB ($n = 4$).

Localization of ENT1 and ENT2 immunoreactivity in cell cultures of dissociated rat carotid body. Using immunofluorescence labeling, we localized ENT expression in permeabilized and non-permeabilized cultures of dissociated rat CB. These cultures consist of isolated type I cell clusters that are easily identified under phase contrast microscopy (Murali and Nurse 2016; Nurse 2010; Salman et al. 2017; Zhang et al. 2012). In permeabilized CB cultures, ENT1 and ENT2 immunoreactivity (green) was found associated with tyrosine hydroxylase (TH)-immunoreactive (ir; red) type I cells as shown in Figs. 5.2a–c and Figs. 5.2d–f, respectively ($n = 2$). Interestingly, ENT1 staining appeared to be mostly localized to the nuclei of TH-positive cells in permeabilized CB cultures as shown in Fig. 5.2a. In non-permeabilized CB cultures, type I cell clusters were also selectively immunopositive for both ENT1 (Fig. 5.2g–i, n = 2) and ENT2 (Fig. 5.2j–l, n = 2), suggesting an additional extracellular localization for both transporters. Note the confinement of ENT1-ir and ENT2-ir to type I cell clusters in Fig. 5.2i, l respectively.

Regulation of ENT1 and ENT2 mRNA expression in rat carotid bodies exposed to chronic hypoxia in vivo.

We recently demonstrated that chronic hypobaric hypoxia *in vivo* up-regulates expression of NTPDase3 and the adenosine producing enzyme, E5'Nt/CD73 in the rat CB (Salman et al. 2017), thereby favoring increased generation of extracellular adenosine. Previous studies showed that chronic hypoxia represses ENT expression in human umbilical vein endothelium (HUVEC) (Casanello et al. 2005) and human microvascular endothelial cells (HMEC) (Eltzschig et al. 2005). We therefore examined whether chronic hypoxia regulates ENT expression in CB, SCG, and PG relative to normoxic controls To this end, we exposed juvenile rat pups (P11-14) to chronic hypobaric hypoxia (60 kPa, simulating an altitude of approximately 4300 m) for 5–7 days as previously described (Salman et al. 2017). For comparison, *quantitative* real time-PCR (qPCR) data adapted from the latter study is plotted in Fig. 5.3, and reveals a significant upregulation in NTPDase3 and E5'Nt mRNAs in the CBs from chronically hypoxic (CHox) animals compared normoxic (Nox) controls in Fig. 5.3. By contrast, similar to previous studies (Casanello et al. 2005; Eltzschig et al. 2005), ENT1 expression was significantly downregulated in the CB and PG of CHox animals relative to Nox controls (Fig. 5.3). ENT2 mRNA was also downregulated and to a lesser extent, though this was specific to the CB tissue of CHox animals (Fig. 5.3). Taken together, these data suggest that chronic hypoxia differentially regulates NTPDase, E5'Nt, and ENT expression in the CB so as to elevate extracellular adenosine levels.

5.4 Discussion

In response to chemostimuli (e.g. hypoxia and acid hypercapnia), ATP is released from the carotid body (CB) and its rapid breakdown to adenosine activates a number of autocrine and paracrine signaling pathways (Buttigieg and Nurse 2004; Conde and Monteiro 2004; Conde et al. 2007) add refs (Murali and Nurse 2016; Nurse and Piskuric 2013; Tse et al. 2012). The main purpose of the present study was to explore mRNA expression pattern and localization of equilibrative nucleoside transporters (ENTs) in the CB and their potential role in adenosine clearance during chronic hypoxia.

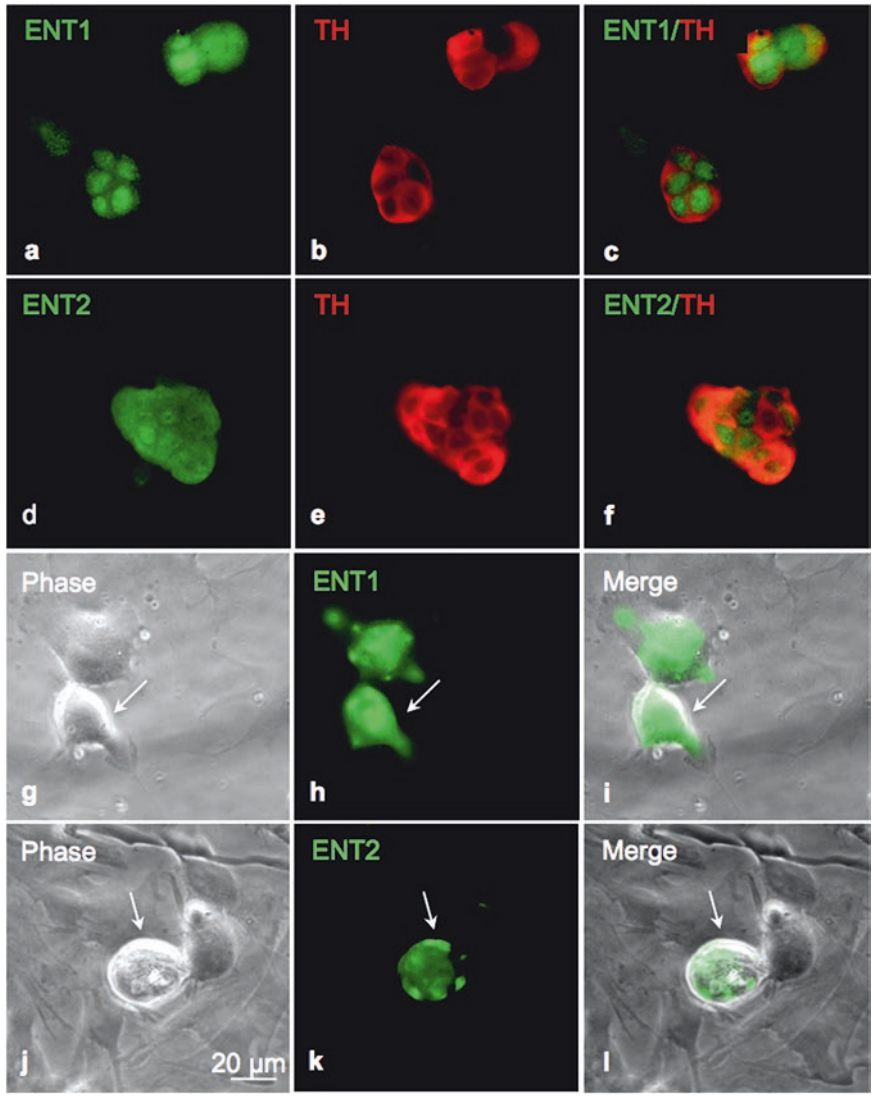

Fig. 5.2 Equilibrative nucleoside transporter1 (ENT1) and ENT2 immunoreactivity in cultures of dissociated rat carotid body. Immunofluorescence staining of permeabilized cultures showing ENT1- and ENT2-ir (green; **a** and **d** respectively) associated within TH-ir (red; **b** and **e**) type I cell clusters; merged image is shown in **c** and **f** (n = 2). *G-I* and *J-L,* ENT1 and ENT2 immunoreactivity in non-permeabilized CB cultures respectively. Note positive extracellular ENT1 and ENT2 immunoreactivity (**h** and **k**) respectively in type I cell clusters (arrow in phase contrast images, **g** and **j**) and merged images in **i** and **l**; note ENT1- and -2 negative background cells surrounding clusters in **i** and **l** (n = 2)

We found that the rat carotid body expresses mRNA and protein for both equilibrative transporter 1 (ENT1) and ENT2. Moreover, mRNA of ENT1 and ENT2 was downregulated in rat CB following *in vivo* exposure to chronic hypobaric hypoxia, and it is proposed that these mechanisms could lead to increased extracellular adenosine concentration at the chemosensory synapse.

Two major metabolic sources for the generation of extracellular adenosine have been identified during mild hypoxia, i.e. ATP catabolism by a series of ectonucleotidase enzymes, and export via the equilibrative nucleoside transport (ENT)

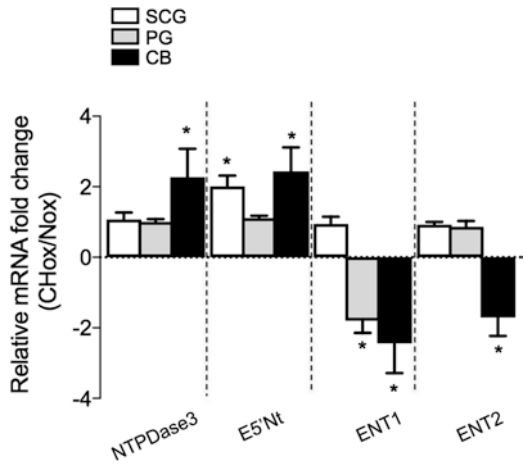

Fig. 5.3 Effects of chronic hypobaric hypoxia on ectonucleoside triphosphate diphosphohydrolase3 (NTPDase3), ecto-5′-nucleotidase (E5′Nt), ENT1 and ENT2 mRNA expression in rat carotid body. Rat pups (~2-week old) were exposed to normoxia or continuous hypobaric hypoxia (60 kPa, ~4300 m) for 5–7 days, starting at day 7. *Quantitative* real-time PCR (qPCR) analysis was used to compare mRNA fold change in chronic hypoxia relative to normoxia of NTPDase3, E5′Nt, ENT1 and ENT2 in SCG, PG and CB tissues. Data reveal a significant upregulation of NTPDase3 and E5′Nt concomitant with a downregulation in ENT1 and ENT2 expression ($n = 4$ litters). Results were normalized to TBP and shown as fold change relative to expression in normoxia. Each experimental replicate represents expression in tissues pooled from individual rat litters (n). Asterisks denote significant differences between normoxic and hypoxic groups, $P < 0.05$

system (Conde et al. 2009). A recent study from our laboratory identified expression of NTPDase1,2,3 and E5′Nt mRNAs in the rat CB, and NTPDase 2,3 and E5′Nt immunoreactivities were localized to type I cell clusters (Salman et al. 2017). The presence of an equilibrative nucleoside transporter (ENT) system in the CB was inferred from pharmacological studies using the ENT inhibitor NBTI (Conde et al. 2008; Conde and Monteiro 2004). Based on those studies it was concluded that ~45% of extracellular adenosine present during mild hypoxia (10% O_2) is trafficked though the ENT system (Conde et al. 2008, Conde and Monteiro 2004). In the present study, we uncovered the molecular identity of the ENT system in the rat CB by showing the rat CB expresses mRNA and protein of ENT1 and ENT2 subtypes. During episodes of high hypoxia intensity, adenosine release by ENT is significantly reduced and extracellular adenosine is preferentially produced by extracellular ATP hydrolysis via the enzymatic action of ectonucleotidases and ecto-5′ nucleotidase (Conde et al. 2012a). The remaining source of adenosine has been proposed to be generated from cAMP (Perez-Garcia et al. 1991).

Previous studies reported transcriptional repression of ENT expression in cardiac myocytes (Chaudary et al. 2004), vascular endothelia and mucosal epithelia (Casanello et al. 2005; Eltzschig et al. 2005) during chronic hypoxia. Given that chronic hypoxia can augment CB chemosensitivity (Kumar and Prabhakar 2012; Powell 2007), and the key role of purinergic signaling in driving the CB afferent discharge (Nurse and Piskuric 2013), it was of interest to contrast ENT expression in CBs isolated from animals exposed to chronic hypobaric hypoxia. Interestingly, we found that chronic hypoxia downregulates mRNA expression of ENT1 and ENT2 in the rat CB, consistent with previous studies reporting a similar downregulation of ENT mRNA in other tissues during chronic hypoxia (Eltzschig et al. 2005).

Adenosine, generated by hydrolysis of extracellular ATP by surface-located enzymes or released through bidirectional equilibrative transporters in type I cells, may activate presynaptic and postsynaptic P1 receptors in the CB (Conde et al. 2009, 2012a; Murali and Nurse 2016; Nurse and Piskuric 2013). In conclusion, the functional consequence of the increased capacity to produce extracellular adenosine from ATP by ectonucleotidases (Conde et al. 2009, 2012a; Gauda 2000; Murali and Nurse 2016; Salman et al. 2017), coupled with the decreased capacity to transport adenosine across type I cells by ENT1,2, predicts a major shift towards enhanced adenosine signaling at the CB chemosensory synapse during chronic hypoxia.

5.5 Statistical Analysis

Statistical analyses were performed using GraphPad Prism7 software. Data are expressed as means ± SE and compared using a non- parametric (Mann-Whitney) test. *n* value indicates the number of litters used in each experiment. Asterisks denote significant differences where $P < 0.05$ between groups.

Acknowledgments We wish to thank Cathy Vollmer for excellent technical assistance and Grant B. McClelland for providing the hypobaric chambers.

References

Bairam A, Niane LM, Joseph V (2013) Role of ATP and adenosine on carotid body function during development. Respir Physiol Neurobiol 185:57–66

Baldwin SA, Beal PR, Yao SY, King AE, Cass CE, Young JD (2004) The equilibrative nucleoside transporter family, SLC29. Pflugers Arch 447:735–743

Baldwin SA, Yao SY, Hyde RJ, Ng AM, Foppolo S et al (2005) Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. J Biol Chem 280:15880–15887

Buttigieg J, Nurse CA (2004) Detection of hypoxia-evoked ATP release from chemoreceptor cells of the rat carotid body. Biochem Biophys Res Commun 322:82–87

Casanello P, Torres A, Sanhueza F, Gonzalez M, Farias M et al (2005) Equilibrative nucleoside transporter 1 expression is downregulated by hypoxia in human umbilical vein endothelium. Circ Res 97:16–24

Chaudary N, Naydenova Z, Shuralyova I, Coe IR (2004) Hypoxia regulates the adenosine transporter, mENT1, in the murine cardiomyocyte cell line, HL-1. Cardiovasc Res 61:780–788

Che M, Ortiz DF, Arias IM (1995) Primary structure and functional expression of a cDNA encoding the bile canalicular, purine-specific Na(+)-nucleoside cotransporter. J Biol Chem 270:13596–13599

Conde SV, Monteiro EC (2004) Hypoxia induces adenosine release from the rat carotid body. J Neurochem 89:1148–1156

Conde SV, Obeso A, Gonzalez C (2007) Low glucose effects on rat carotid body chemoreceptor cells' secretory responses and action potential frequency in the carotid sinus nerve. J Physiol 585:721–730

Conde SV, Gonzalez C, Batuca JR, Monteiro EC, Obeso A (2008) An antagonistic interaction between A2B adenosine and D2 dopamine receptors modulates the function of rat carotid body chemoreceptor cells. J Neurochem 107:1369–1381

Conde SV, Monteiro EC, Obeso A, Gonzalez C (2009) Adenosine in peripheral chemoreception: new insights into a historically overlooked molecule--invited article. Adv Exp Med Biol 648:145–159

Conde SV, Monteiro EC, Rigual R, Obeso A, Gonzalez C (2012a) Hypoxic intensity: a determinant for the contribution of ATP and adenosine to the genesis of carotid body chemosensory activity. J Appl Physiol 1985(112):2002–2010

Conde SV, Ribeiro MJ, Obeso A, Rigual R, Monteiro EC, Gonzalez C (2012b) Chronic caffeine intake in adult rat inhibits carotid body sensitization produced by chronic sustained hypoxia but maintains intact chemoreflex output. Mol Pharmacol 82:1056–1065

Connolly GP, Stone TW, Brown F (1993) Characterization of the adenosine receptors of the rat superior cervical ganglion. Br J Pharmacol 110:854–860

Eltzschig HK, Abdulla P, Hoffman E, Hamilton KE, Daniels D et al (2005) HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. J Exp Med 202:1493–1505

Gauda EB (2000) Expression and localization of A2a and A1-adenosine receptor genes in the rat carotid body and petrosal ganglia. A2a and A1-adenosine receptor mRNAs in the rat carotid body. Adv Exp Med Biol 475:549–558

Gonzalez C, Almaraz L, Obeso A, Rigual R (1994) Carotid body chemoreceptors: from natural stimuli to sensory discharges. Physiol Rev 74:829–898

Govindarajan R, Leung GP, Zhou M, Tse CM, Wang J, Unadkat JD (2009) Facilitated mitochondrial import of antiviral and anticancer nucleoside drugs by human equilibrative nucleoside transporter-3. Am J Physiol Gastrointest Liver Physiol 296:G910–G922

Holmes AP, Ray CJ, Pearson SA, Coney AM, Kumar P (2017) Ecto-5′-nucleotidase (CD73) regulates peripheral chemoreceptor activity and cardiorespiratory responses to hypoxia. J Physiol

Kobayashi S, Zimmermann H, Millhorn DE (2000) Chronic hypoxia enhances adenosine release in rat PC12 cells by altering adenosine metabolism and membrane transport. J Neurochem 74:621–632

Kumar P, Prabhakar NR (2012) Peripheral chemoreceptors: function and plasticity of the carotid body. Compr Physiol 2:141–219

Lopez-Barneo J, Ortega-Saenz P, Pardal R, Pascual A, Piruat JI (2008) Carotid body oxygen sensing. Eur Respir J 32:1386–1398

Murali S, Nurse CA (2016) Purinergic signalling mediates bidirectional crosstalk between chemoreceptor type I and glial-like type II cells of the rat carotid body. J Physiol 594:391–406

Nurse CA (2010) Neurotransmitter and neuromodulatory mechanisms at peripheral arterial chemoreceptors. Exp Physiol 95:657–667

Nurse CA (2014) Synaptic and paracrine mechanisms at carotid body arterial chemoreceptors. J Physiol 592:3419–3426

Nurse CA, Piskuric NA (2013) Signal processing at mammalian carotid body chemoreceptors. Semin Cell Dev Biol 24:22–30

Peers C, Buckler KJ (1995) Transduction of chemostimuli by the type I carotid body cell. J Membr Biol 144:1–9

Perez-Garcia MT, Almaraz L, Gonzalez C (1991) Cyclic AMP modulates differentially the release of dopamine induced by hypoxia and other stimuli and increases dopamine synthesis in the rabbit carotid body. J Neurochem 57:1992–2000

Powell FL (2007) The influence of chronic hypoxia upon chemoreception. Respir Physiol Neurobiol 157:154–161

Prasad M, Fearon IM, Zhang M, Laing M, Vollmer C, Nurse CA (2001) Expression of P2X2 and P2X3 receptor subunits in rat carotid body afferent neurones: role in chemosensory signalling. J Physiol 537:667–677

Rong W, Gourine AV, Cockayne DA, Xiang Z, Ford AP et al (2003) Pivotal role of nucleotide P2X2 receptor subunit of the ATP-gated ion channel mediating ventilatory responses to hypoxia. J Neurosci 23:11315–11321

Salman S, Vollmer C, McClelland GB, Nurse CA (2017) Characterization of ectonucleotidase expression in the rat carotid body: regulation by chronic hypoxia. Am J Physiol Cell Physiol 313:C274–CC84

Tse A, Yan L, Lee AK, Tse FW (2012) Autocrine and paracrine actions of ATP in rat carotid body. Can J Physiol Pharmacol 90:705–711

Xu J, Tse FW, Tse A (2003) ATP triggers intracellular Ca2+ release in type II cells of the rat carotid body. J Physiol 549:739–747

Zhang M, Zhong H, Vollmer C, Nurse CA (2000) Co-release of ATP and ACh mediates hypoxic signalling at rat carotid body chemoreceptors. J Physiol 525(Pt 1):143–158

Zhang M, Piskuric NA, Vollmer C, Nurse CA (2012) P2Y2 receptor activation opens pannexin-1 channels in rat carotid body type II cells: potential role in amplifying the neurotransmitter ATP. J Physiol 590:4335–4350