Chapter 5
Targeting Carbonic Anhydrase Isozymes in the Treatment of Neurological Disorders

Ashok Aspatwar, Jukka Peltola, and Seppo Parkkila

Abstract Carbonic anhydrases (CAs) are widely expressed in the nervous system where they play important physiological roles. In the brain and other parts of the system, different isozymes show unique distribution patterns, some of them being present in neurons (CA II, V, VII, XIV), capillary endothelium (CA IV), microglia (CA III), choroid plexus (CA II, III, XII, XIV), astrocytes (CA II and V), oligodendrocytes (CA II and XIII), and myelin sheath (CA II). Nervous tissues also express three carbonic anhydrase-related proteins (CARP VIII, X, XI), which may be involved in the brain development processes. Future research is needed to define the exact roles of these highly conserved CA isoforms and to design novel treatment strategies for the diseases caused by defects or abnormal regulation of CARPs. Enzymatically active CA isozymes are known drug targets to treat various neurological disorders including epilepsy, acute mountain sickness, pseudotumor cerebri, and brain edema. In this review article, we describe how the clinically approved CA inhibitors are used for the treatment of these diseases.

Keywords Brain · Carbonic anhydrase · Drug · Expression · Inhibitor · Neurology

5.1 Expression and Function of Enzymatically Active Carbonic Anhydrases in the Nervous System

The presence of carbonic anhydrase (CA) activity in the brain was described for the first time in 1943 (Ashby 1943). In the nervous system, CAs play various important roles, e.g., in fluid and ion compartmentation (Bourke and Kimelberg 1975), the formation of cerebrospinal fluid (CSF) (Maren 1967), neuronal signal transduction

A. Aspatwar · J. Peltola · S. Parkkila (✉)
Faculty of Medicine and Health Technology, Tampere University, Arvo Ylpön katu 34, 33520 Tampere, Finland
e-mail: seppo.parkkila@tuni.fi

J. Peltola · S. Parkkila
Fimlab Ltd. and Department of Neurology, Tampere University Hospital, Tampere, Finland

© Springer Nature Switzerland AG 2021

W. R. Chegwidden and N. D. Carter (eds.), *The Carbonic Anhydrases: Current and Emerging Therapeutic Targets*, Progress in Drug Research 75,
https://doi.org/10.1007/978-3-030-79511-5_5

Table 5.1 Distribution of CA isozymes in different cell types of the central nervous system

Cell type	Isozymes
Oligodendrocytes	II, XIII (Roussel 1979; Ghandour 1980,1979; Langley 1980; Kumpulainen and Korhonen 1982; Kumpulainen 1983; Kida 2006; Lehtonen 2004)
Astrocytes	II, V (Cammer 1991; Kimelberg et al. 1982; Snyder 1983; Ghandour 2000; Cammer and Tansey 1988a,b; Cammer and Zhang 1992; Jeffrey et al. 1991)
Myelin sheath	II (Roussel 1979; Kumpulainen and Korhonen 1982)
Choroid plexus	II, III, XII, XIV (Kumpulainen and Korhonen 1982; Kida 2006; Nogradi et al. 1993; Parkkila 2001; Kallio 2006; Halmi et al. 2006)
Microglial cells	III (Nogradi 1993)
Endothelial cells	IV (Ghandour 1992)
Neurons	II, V, VII, XIV (Ruusuvuori 2004; Kida 2006; Parkkila 2001; Ghandour 2000)

(Ruusuvuori 2004; Makani 2012), seizure activity (Anderson et al. 1984), the respiratory response to carbon dioxide (Ridderstrale and Hanson 1985), and the generation of bicarbonate for biosynthetic reactions (Cammer 1991).

The distribution of various CA isozymes in the brain is summarized in Table 5.1. In an early histochemical study, the highest CA activity was shown in the areas of mouse brains that were rich in myelinated fibers and glial cells (Korhonen et al. 1964). Several early immunohistochemical studies concluded that the mammalian brain contains CA mainly or exclusively in oligodendrocytes (Roussel 1979; Ghandour 1980,1979; Langley 1980; Kumpulainen and Korhonen 1982; Kumpulainen 1983). Myelin sheaths were also found to contain CA II (Roussel 1979; Kumpulainen and Korhonen 1982), which prompted the idea that CA II could be a biomarker for myelin degradation in demyelinating diseases (Kumpulainen 1985; Parkkila 1997).

The expression of CA II in astrocytes has been a controversial matter, and some findings suggested that these cells may contain CA II at relatively low levels (Roussel 1979; Kimelberg et al. 1982; Snyder 1983). The presence of CA II in astrocytes was questioned since in situ hybridization showed CA II mRNA expression in oligodendrocytes but not in astrocytes (Ghandour and Skoff 1991). Neurons have generally been considered to lack CA II, but there have been some exceptions to this notion, and obviously some subpopulations of neurons may express it (Kida 2006; Tanimoto et al. 2005).

Nogradi demonstrated CA II and CA III immunoreactivity in active brain macrophages, whereas resting microglial cells expressed only CA III (Nogradi 1993). The changes in CA II expression that occur in microglial cells during development and activation may correlate with the metabolic and immunological states of the cells.

The choroid plexus is one of the main sites of CA expression within the central nervous system, and the significance of CA activity for CSF formation is well documented (Maren 1967; Tschirgi et al. 1954; Brown 2004). CA II and CA III were found in the epithelial cells of the choroid plexus by Kumpulainen and Korhonen

(1982) and Nogradi et al., respectively (1993). In addition to these cytoplasmic forms, the choroid plexus contains at least three membrane-associated isozymes. First, CA XIV was found in a limited population of choroid plexus epithelial cells (Parkkila 2001). Another immunohistochemical study demonstrated strong expression of both CA IX and CA XII in the choroid plexus (Ivanov 2001). Later, the positive signal for CA XII was located to the basolateral membranes of the choroid plexus epithelial cells (Kallio 2006), and a role for CA XII in the recycling of carbon dioxide into the epithelial cells was proposed (Halmi et al. 2006).

The membrane-associated CA IV has been demonstrated on the luminal surface of capillary endothelial cells (Ghandour 1992). The strategic location on the luminal face of the blood-brain barrier suggests that CA IV may participate in the export of carbon dioxide from brain tissues. In 2006, it was shown that CA IV is expressed on the surfaces of astrocytes (Svichar 2006). Using CA IV and XIV double knockout mice, it was observed that both membrane-associated CAs contribute to extracellular buffering in the central nervous system, and CA IV seems to be the more important extracellular CA in the hippocampus (Shah 2005; Svichar 2009). It has been shown that thyroid hormone signaling regulates the expression of CA IV in the brain (Vujovic 2015).

There have been very few studies on the expression and role of mitochondrial CA VA and VB in the nervous system. In the first paper, Ghandour and coworkers demonstrated that the mitochondrial enzyme is present in both astrocytes and neurons (Ghandour 2000). At that moment, it was not known whether the neuronal isozyme was CA VA or CA VB. Western and Northern blot experiments later confirmed that the isozyme was indeed CA VB, which generally shows a wider expression pattern in tissues compared to CA VA (Shah 2000). Several potential functions have been proposed for the mitochondrial enzyme (Ghandour 2000): It could play a role in gluconeogenesis in astrocytes by providing bicarbonate ions for the pyruvate carboxylase, the neuronal CA V could be involved in the regulation of the intramitochondrial calcium levels, and it could also participate in bicarbonate ion-induced GABA responses by regulating the bicarbonate homeostasis in neurons. Kaila´s group later reported that it is the other isozyme, CA VII, that is the key molecule in the generation of high-frequency stimulation-induced tonic GABAergic excitation (Ruusuvuori 2004).

Although the expression of CA IX seems quite limited and generally weak in the central nervous system, it may still carry out important functions in the regulation of behavior and maintenance of tissue integrity. Preliminary observations suggested mild behavioral changes and a morphological disruption of brain histology in CA IX-deficient (*Car9* (-/-)) mice. Therefore, a one-year follow-up study was conducted in which both the behavior and brain histology of *Car9* (-/-) and wild-type mice were monitored (Pan 2012). Morphological analysis revealed vacuolar degenerative changes in the brains of *Car9* (-/-) mice. The changes became visible at the age of eight to ten months. Behavioral tests showed that the *Car9* (-/-) mice exhibited abnormal locomotor activity and poor performance in a memory test. To further identify the transcriptomic responses to CA IX deficiency in the brain, genome-wide cDNA microarray analyses were performed. Functional annotation revealed

that the genes with increased expression were involved in several processes, such as RNA metabolism, and the genes with reduced expression were implicated in other important processes, including the regulation of cellular ion homeostasis. Notably, the biological processes "behavior" and "locomotory behavior" were the two prominent terms overrepresented among the downregulated genes.

The distribution and role of CA XIII are poorly known at this moment. The first article on CA XIII showed a positive mRNA signal in the mouse brain and located the enzyme to the oligodendrocytes and nerve fibers (Lehtonen 2004).

5.2 CA Inhibitors in the Treatment of Neurological Diseases

The classical CA inhibitor acetazolamide is clinically used for the treatment of acute high-altitude illness (AHAI), brain edema, pseudotumor cerebri, and epilepsy, in addition to the nonneurological indications. This inhibitor has also been tested for the treatment of hydrocephalus because there are no significant noninvasive treatment alternatives (Groat and Neumiller 2013), and the reduction of CSF production would be of interest as a treatment strategy. Some studies have indeed shown that both acetazolamide and a well-known diuretic, furosemide, could be useful to reduce CSF production by the choroid plexus. As with lumbar puncture, these agents are used in low-birthweight infants who will have a low success rate with shunt placement or endoscopic ventriculostomy. There is no evidence that either of these medications increases survival rates, and a Cochrane review concluded that therapy with acetazolamide or furosemide is neither effective nor safe for treating posthemorrhagic ventricular dilatation in infants (Whitelaw et al. 2001).

5.3 CA Inhibitors in the Treatment of Acute High-Altitude Illness

Acute high-altitude illness (AHAI) is an encompassing term for the range of pathology that the unacclimatized individual may develop when exposed to hypoxia at high altitude (Smedley and Grocott 2013). The term AHAI covers acute mountain sickness (AMS), high-altitude cerebral edema (HACE) and high-altitude pulmonary edema (HAPE). The symptoms of AMS include headache, dizziness, nausea, insomnia, anorexia, and difficulty sleeping. Progression to HACE is characterized by altered mental status, reduced consciousness and ataxia.

Acetazolamide is considered one of the key medications for both AMS prevention and treatment and can also be used as an adjunct to dexamethasone in HACE treatment (Table 5.2) (Luks 2010). The severity of additional diseases of high altitude may also be reduced by acetazolamide, including HAPE and chronic mountain sickness (CMS) (Swenson 2014). It is noteworthy that the beneficial effect of acetazolamide

Table 5.2 Recommended medications used in the prevention and treatment of AMS and HACE (Luks 2010)

Medication	Indication	Route	Dosage in adults
Acetazolamide	AMS, HACE prevention	Oral	125 mg twice per day
	AMS, HACE[a] treatment	Oral	250 mg twice per day
Dexamethasone	AMS, HACE prevention	Oral	2 mg every 6 h or 4 mg every 12 h
	AMS, HACE treatment	Oral, IV, IM	AMS: 4 mg every 6 h HACE: 8 mg once, then 4 mg every 6 h

IV intravenous; *IM* intramuscular; [a]Acetazolamide can be used at this dose as an adjunct to dexamethasone in HACE treatment, but dexamethasone remains the primary treatment for that disorder

in high-altitude illness may not be related to the reduction of CSF production only. It has been shown that acetazolamide also blocks aquaporin-4 (AQP4) water channels (Huber 2007), the main component of the glial-associated lymphatic pathway called the glymphatic system (Plog and Nedergaard 2018). Therefore, it is obvious that acetazolamide can reduce the swelling of the brain tissue in the hypobaric hypoxia condition by modulation of the glymphatic system.

5.4 CA Inhibitors in the Treatment of Pseudotumor Cerebri

Pseudotumor cerebri is a neurological disease where intracranial pressure increases for no obvious reason. Symptoms mimic those of a brain tumor, even though no tumor is present. When no underlying cause of the increased intracranial pressure is discovered, pseudotumor cerebri may also be called idiopathic or benign intracranial hypertension. It is almost exclusively a disease of obese young women (Wall 2014). The increased intracranial pressure associated with pseudotumor cerebri can cause swelling of the optic nerve and result in vision loss. The key factor for the pathogenesis of pseudotumor cerebri is the accumulation of CSF. This may be caused by either increased fluid production or decreased fluid reabsorption. Several CAs are expressed in the choroid plexus (Table 5.1), the site of CSF production. Therefore, it was reasonable to consider CA inhibitors as potential treatments for pseudotumor cerebri. In fact, it was first documented in the 1950s that acetazolamide reduces intracranial pressure (Atkinson and Ward 1958). Since then, it has been widely used in the first-line therapy of pseudotumor cerebri, although solid documentation of the effectiveness was lacking for many years (Thurtell and Wall 2013). A large, multicenter, randomized controlled trial was conducted in 2010–2014, comparing the efficacy of weight loss and placebo with weight loss and acetazolamide as treatments for mild to moderate idiopathic intracranial hypertension (https://clinicaltrials.gov/ct2/

show/NCT01003639). The main result indicated that the use of acetazolamide with a low-sodium weight-reduction diet compared with diet alone resulted in modest improvement in the visual field function of patients with idiopathic intracranial hypertension and mild vision loss (Committee et al. 2014). There is no standardized dosing regimen for acetazolamide as a treatment of pseudotumor cerebri. It has been suggested that a reasonable starting dose could be 500 mg twice daily, gradually increasing to a maximum of 4 g daily in twice-daily doses depending on the treatment outcome and potential side effects of the drug (Thurtell and Wall 2013). Methazolamide, another CA inhibitor, can be tried when the side effects of acetazolamide are intolerable (Thurtell and Wall 2013). In a recent review, Supuran predicted that no major changes would occur in the use of acetazolamide in the treatment of intracranial hypertension within the near future because it is a safe, nontoxic and inexpensive drug that is not protected by any patents (Supuran 2015).

Topiramate, a widely used anticonvulsant, is the third potential CA inhibitor that has been proposed as a treatment of pseudotumor cerebri (Thurtell and Wall 2013). It has a clear anti-obesity effect causing dose-dependent weight loss (Ben-Menachem 2003; Bray 2003), which is considered a desired outcome in the treatment of pseudotumor cerebri. Compared to acetazolamide, topiramate seems to show similar efficacy for improvement of visual field grades (Celebisoy 2007).

5.5 CA Inhibitors in the Treatment of Brain Edema

As discussed above, acetazolamide is clinically used to reduce intracranial pressure due to pseudotumor cerebri. Even though it has been used much less in other forms of neurological/neurosurgical diseases associated with increased intracranial pressure, it has been successfully applied in certain neurosurgical conditions for diagnostic or therapeutic purposes. Acetazolamide is anti-edematous and has neuroprotective properties that have been discussed in the literature (Szczygielski 2019).

Mild traumatic brain injury is a relatively common disease entity experienced in accidents, on the battlefield, and in sports. Astrocyte cellular edema, where AQP4 is involved, is an important factor leading to high morbidity after brain injury. As a blocking agent of AQP4, acetazolamide could represent a potential drug for the treatment of mild traumatic brain injury. Sturdivant and coworkers recently investigated whether acetazolamide could prevent AQP4-driven cell swelling using a 3D astrocyte model of mild traumatic brain injury (Sturdivant 2016). First, they proved that AQP4 expression was significantly increased 24 h after the procedure, mimicking mild traumatic brain injury. The procedure resulted in an increase in cell swelling within 30 min of mild traumatic brain injury, which was significantly reduced in the presence of acetazolamide. Cell death was also significantly reduced when acetazolamide was added shortly before the trauma procedure, supporting the neuroprotective role of the drug. To date, there are no clinical studies addressing the use of acetazolamide in this indication.

5.6 CA Inhibitors in the Treatment of Epilepsy

Epilepsy is a very common neurological disease, and seizures are possible in any individual in response to an appropriate stimulus. Different stimuli include lack of sleep, smoking, and reduced CO_2 levels in the brain (Aggarwal et al. 2013). Since CO_2 is involved, several CA inhibitors have been considered potential candidates for the treatment of seizures, as they could increase the CO_2 levels in the brain. In fact, CA inhibitors have been used as antiepileptic drugs (AEDs) in the 1970s, when acetazolamide and methazolamide were the most promising choices (Supuran 2018). The mechanisms by which CA inhibitors show antiepileptic action are complex. The inhibition of CA activity leads to a diminished formation of bicarbonate and changes the brain pH, thus contributing to an antiepileptic effect by several diverse pathways. However, the clinical significance of the inhibition of CA in the treatment of epilepsy is not well established, except in the case of acetazolamide, mainly because most of the AEDs in clinical usage with CA inhibition properties also have other, most likely more important, mechanisms of action.

Acetazolamide was the first CA inhibitor used as an anticonvulsant. Because of its numerous side effects, including alterations of taste, paresthesia, and tinnitus, acetazolamide is currently rarely used for this indication (Aggarwal et al. 2013). Catamenial epilepsy is a special subtype of epilepsy in which seizures are clustered around specific points of the menstrual cycle, most often around the perimenstrual or periovulatory period. Three types of catamenial seizures have been identified: perimenstrual (C1), periovulatory (C2), and inadequate luteal (C3) (Navis and Harden 2016). Acetazolamide has been used for decades as a treatment option in catamenial epilepsy, but there are no large-scale, randomized studies available on its efficacy (Aggarwal et al. 2013). In a retrospective analysis, 40% of the women patients reported lower seizure frequency, and 30% claimed a decrease in severity with acetazolamide treatment (Lim 2001). Based on previous studies and present treatment options, acetazolamide is still considered part of the algorithm for the treatment of female patients with suspected catamenial seizure patterns (Navis and Harden 2016).

AEDs with CA inhibitory characteristics, which are commonly used in the treatment of epilepsy, include topiramate, zonisamide and lacosamide. In the case of these three AEDs, CA inhibition has not been considered the defining characteristic as a mechanism of action for efficacy in the treatment of various forms of epilepsy, but it has relevance with regard to their side-effect profiles, especially with topiramate and zonisamide.

Topiramate is a sulfamate-substituted monosaccharide that was originally found to exhibit potent anticonvulsant activity similar to phenytoin (Maryanoff et al. 1987). In the first report, topiramate was described as a weak CA inhibitor (micromolar against erythrocyte CAs), but later Supuran and coworkers demonstrated that topiramate is, in fact, a very potent inhibitor of CAs with a K_i value of 5 nM against human CA II (Masereel 2002). Topiramate inhibits all CA isozymes present in the blood and brain, causing CO_2 retention, which is considered important for the anticonvulsant effect (Aggarwal et al. 2013). Other mechanisms may also be involved, such as the

blockade of Na^+ channels and AMPA/kainate receptors, as well as the enhancement of GABAergic transmission.

Zonisamide is a sulfonamide antiepileptic drug that is a 1,2 benzisoxazole derivative with multiple mechanisms of action resembling topiramate. It was first used in Japan in 1972 to treat psychiatric disorders, and it has been in use to treat epilepsy since at least the 1990s.

Topiramate and zonisamide have a number of similar side effects attributed to CA inhibition. The most common of these are hypohidrosis (Cheshire and Fealey 2008), tubular acidosis and renal stones (Hamed 2017). Hypohidrosis signifies decreased sweating in response to a proportionate thermal or pharmacological stimulus with its complete form termed anhidrosis. Hypohidrosis is potentially hazardous to health since the inability to generate a thermoregulatory sweating response can seriously challenge one's ability to maintain core temperature in conditions of strenuous physical activity or in hot environments (Cheshire and Fealey 2008). CA inhibitors can interfere with sweat production, probably at the level of the secretory coil clear cell or apex of ductal cells. The risk of hypohidrosis in pediatric patients taking zonisamide or topiramate is significantly higher than that of adults (Cheshire and Fealey 2008). Hypohidrosis has been reported mainly during adjunctive topiramate therapy and is rare in patients on monotherapy (Cerminara 2006). When children who are severely disabled (i.e., neurologically impaired, nonambulatory) are treated with topiramate, they have a very high risk of renal stone formation (Ishikawa 2019).

In addition to epilepsy, zonisamide has been tested for the treatment of other symptoms and diseases, such as essential tremor, myoclonus-dystonia, mania, acute psychotic conditions, neuropathic pain, and Parkinson's disease symptoms (Kadian et al. 2019). A Cochrane review on essential tremor identified only one study with 20 eligible subjects (Bruno et al. 2017). The main conclusion was that there is insufficient evidence to assess the efficacy and safety of zonisamide for the treatment of essential tremor. In 2016, Hainque and coworkers investigated the efficacy and safety of zonisamide in a cohort of 24 patients with myoclonus-dystonia (Hainque 2016). Zonisamide significantly improved action myoclonus, myoclonus-related functional disability, and dystonia symptoms compared to the placebo.

Lacosamide, (2*R*)-2-acetylamino-*N*-benzyl-3-methoxypropanamide, is a relatively new antiepileptic drug that was first introduced into clinical practice in 2008. Although it does not have any of the moieties typically found in well-known CA inhibitors, such as sulfonamide, sulfamate, sulfamide or coumarin, lacosamide acts as an effective inhibitor of all mammalian CA isozymes CA I − XV. Based on crystallographic data, it binds to the active site of CA II similar to the hydrolyzed coumarins, not interacting with the metal ion (Temperini 2010). Experimental data have suggested a dual mechanism of action for lacosamide: (a) modulation of the slow inactivation of sodium channels and (b) modulation of collapsin-response mediator protein 2 (CRMP-2)-mediated neurotrophic signals (Kellinghaus 2009). It seems that the inhibition of CA activity may be the third relevant function, which may lead to the antiepileptic effect. In contrast to topiramate or zonisamide, lacosamide does not seem to have hypohidrosis, tubular acidosis or renal stones as part of its side effect profile (Kwok et al. 2017).

5.7 Carbonic Anhydrase-Related Proteins (CARPs)

The carbonic anhydrase-related proteins (CARPs) are CA isoforms that are evolutionarily well conserved but lack the classical CA enzymatic activity (Aspatwar 2014; Aspatwar et al. 2010). The CARPs occur either independently or as domains of other proteins. The catalytic inactivity of CARPs is due to the absence of one or more of the three histidine residues that are required for the coordination of zinc metal ions in the CA active site. There are three classical CARPs named CARP VIII, X and XI in the order of their discovery. In addition, in the family of protein tyrosine phosphatases (PTPs), there are two receptor-type protein tyrosine phosphatases, PTPR zeta (ζ) and PTPR gamma (γ), that contain an N-terminal CA-like domain (Barnea 1993; Levy 1993) known as the CARP XVI domain (Tolvanen 2012; Ortutay et al. 2010). The enzymatic activity of human CARPs can be regained by restoring the histidine residues (Nishimori 2013). Even though we have gradually learned more about the potential biological functions of CARPs and CA domains, the exact physiological roles of these proteins are still poorly understood.

5.8 Localization and Role of CARP VIII

CARP VIII is catalytically inactive due to the substitution of arginine at the first of the three histidine residues required for coordination of the zinc atom in the active site. CARP VIII was the first CARP isoform that was identified and found in the mouse brain by Kato (1990). Subsequent expression studies included detailed analyses during embryonic development and of adult tissues of humans and mice (Aspatwar et al. 2010; Taniuchi 2002a,b; Lakkis 1997). The expression of the *Car8* gene was studied using in situ hybridization, which localized the *Car8* gene to the cerebellar Purkinje cells (Lakkis et al. 1997). Immunohistochemistry showed that CARP VIII is expressed in both adult and fetal human brains, and the cellular distribution of the protein is shown in Table 5.3 (Taniuchi 2002b). The analysis of *CA8* gene expression in a panel of zebrafish tissues showed that it is predominantly expressed in the brain, similar to the expression in mice and humans (Aspatwar et al. 2010; Okamoto 2001; Aspatwar 2013). In the adult zebrafish tissues, immunohistochemistry of the cerebellar region showed an intense signal for CARP VIII in the Purkinje cells, which is analogous to the expression in mice and humans (Aspatwar 2014,2015).

The role of CARP VIII in neural development became obvious from the studies in waddles (*wdl*) mice that are characterized by a lifelong gait disorder (Jiao 2005). Analysis of *wdl* mice showed a 19 bp deletion in the *Car8* gene (autosomal recessive mutation), which is responsible for the *wdl* phenotype, and the mice showed a complete absence of CARP VIII protein (Jiao 2005).

Along with two reports on *CA8* gene mutations in members of Iraqi and Saudi Arabian families, the role of CARP VIII in neural development has gained renewed interest (Turkmen 2009; Kaya 2011). The affected members of an Iraqi family had

Table 5.3 Expression of CARPs in human adult and fetal brains (Aspatwar et al. 2013)

Adult brain					Fetal brain			
		CARP VIII	CARP X	CARP XI	Days of gestation	CARP VIII	CARP X	CARP XI
Brain part	Region	Level of expression				Level of expression		
Cerebrum	Cx	++	+w	+	84	+	+	+
	Medulla	+	++	−	95	+	+	+
	Hippocampus	++	−	+	121	+	+	+
	Basal ganglia	++	−	+w	141	+	+	+
Diencephalon	Thalamus	++	−	−	222	+	+	+
	Substantia nigra	++	−	−				
Cerebellum	Cx, molecular layer	++	−	−				
	Cx, Purkinje cells	++	+w	+w				
	Cx, granular layer	−	−	−				
Pons	Vestibular nuclei	++	−	+				
	Abducens nucleus	++	−	+				
	Pontine nuclei	++		++				
Medulla	Olivary nuclei	++	+w	−				
	Others	++	+w	−				
Choroid PX		++	+w	++				
Pia Arach		++	−	++				

++ strong expression; + moderate expression in most cells; +*w* weak expression; − no significant expression; *Cx* cortex; *Choroid PX*. choroid plexus; *Pia Arach.* pia arachnoid

mild mental retardation and congenital ataxia that was associated with quadrupedal gait (Fig. 5.1). In another study, the members of a Saudi Arabian family showed a novel homozygous (G162R) substitution in the CARP VIII protein (Kaya 2011). All the affected members of the family showed mental retardation and cerebellar ataxia similar to the affected members of the Iraqi family, but the Saudi Arabian patients did not exhibit quadrupedal gait (Aspatwar et al. 2013). Brain magnetic resonance imaging showed a loss of cerebellar volume and peritrigonal white matter abnormalities (Kaya 2011). Mild cognitive impairment, a variable degree of cerebellar ataxia, an absence of seizures, and a lack of dysmorphism were the common features that were found in both Saudi Arabian and Iraqi subjects. The absence of quadrupedal gait

Figure. 5.1 A mutation in the *CA8* gene in members of an Iraqi family leads to mental retardation and quadrupedal gait. This figure is reproduced from Turkmen (2009) under the Creative Commons Attribution (CC BY) license

in the members of the Saudi Arabian family could be due to environmental factors rather than being a genuine phenotypic variation of the disease.

A report on the interaction of CARP VIII with inositol trisphosphate receptor-1 (ITPR1) provides a plausible mechanism for the cerebellar disorders in humans and mice (Hirota 2003; Yan 2007). However, the precise mechanism of how the regulation of ITPR1 leads to the biological effects is still not known. Later studies in mice showed that *Car8* acts as an allosteric inhibitor of ITPR1 that regulates the intracellular calcium release essential for neuronal excitability, neurite outgrowth, neurotransmitter release (Lamont and Weber 2015), and the calcium pathway that is critical for nociception, inflammatory pain, and possibly other neuropathological states (Zhuang 2015).

Changes in the gene expression profile of human neuroblastoma cells expressing a repeat expansion mutant ataxin-3 showed a ninefold increase in *CA8* gene expression in the presence of mutant ataxin-3 compared to the cells expressing normal ataxin-3 (Hsieh 2012). It was shown earlier that defective ataxin-3 disturbs neuronal calcium signaling by binding to ITPR1 (Chen 2008). There are now many lines of evidence suggesting that CARP VIII plays a role in neural development and motor coordination function in the cerebellum (Shimobayashi et al. 2016).

To further elucidate the function of CARP VIII, we recently developed a zebrafish model by knocking down the *CA8* gene using antisense morpholino oligonucleotides (Aspatwar 2013). The amino acid identity between the human and zebrafish CARP VIII protein is 84%. Such a high degree of conservation between CARP VIII sequences of distant vertebrate species speaks for a conserved, essential function

(Aspatwar 2013). The developed zebrafish model will be helpful in investigating the mechanisms of CARP VIII-related ataxia and mental retardation in humans.

5.9 Distribution and Role of CARP X

The presence of CARP X was reported in the brain while screening CCG repeats in the cDNA libraries from the human brain (Okamoto 2001). The deduced CA-like amino acid sequence showed that two of the three amino acid residues required for binding to zinc were absent in CARP X, suggesting the lack of enzymatic activity (Aspatwar et al. 2013). *CA10* mRNA expression was reported in all parts of the central nervous system analyzed (Okamoto 2001). The studies of the cellular distribution of CARP X using antibodies also showed positive signals in many parts of the human brain (Table 5.3) (Taniuchi 2002b; Aspatwar et al. 2013). A developmental expression study in human brain specimens covering days 84–222 of gestation showed CARP X protein throughout the period (Taniuchi 2002b). In our laboratory, we showed the presence of *Car10* mRNA extensively in the mouse brain (Aspatwar et al. 2010), and similarly, *CA10* was predominantly expressed in both the larval and adult zebrafish brains (Aspatwar 2015). Knockdown studies of *CA10a* and *CA10b* genes using morpholinos in zebrafish larvae showed an abnormal movement pattern, which was later confirmed by inactivation of the genes with the CRISPR/Cas9 system, suggesting a role for these CARPs in motor coordination (Aspatwar 2015).

Recent studies have shown that both CARP X and XI are evolutionarily conserved neurexin ligands in mammals (Sterky 2017). Neurexins are presynaptic proteins that regulate neurotransmitter release. Notably, damage to these synapses precedes neuronal cell death in Alzheimer's disease (Hishimoto 2019). The above results have led to several persuasive hypotheses. First, CARP X and CARP XI may act primarily as neurexin chaperones. Second, they may function as adaptors that enable indirect interactions of neurexins with unknown postsynaptic target molecules, thus mediating the formation of novel transsynaptic complexes (Sterky 2017).

The presence of seven CCG repeats in the 5'-untranslated region of the *CA10* gene followed by two CCG repeats located 16 bp downstream may be associated with various neurological disorders (Kleiderlein 1998). The *CA10* gene with the CCG repeats might play a role in the development of neurodegenerative disorders, and therefore, it will be of interest to explore the expansion mutations of the *CA10* gene in patients with neurological symptoms (Aspatwar et al. 2010). It is of interest that there is a report on cerebellar hypoplasia and quadrupedal movement in members of a Turkish family (Turkmen 2006; Humphrey and Mondalas 2018) who showed a quite similar phenotype to the Iraqi and Saudi Arabian patients with mutations of the *CA8* gene (Turkmen 2009; Kaya 2011). However, the genetic defect in the Turkish family was mapped to chromosome 17p (Turkmen 2006; Humphrey and Mondalas 2018). Because the *CA10* gene is located on chromosome 17q, it may not be the affected gene in this phenotypic trait.

5.10 Distribution and Role of CARP XI

A characteristic feature of CARP XI is the absence of all three histidine residues required for the CA catalytic activity (Aspatwar et al. 2010). The cellular and developmental distribution of the human CARP XI protein is shown in Table 5.3. The expression of *Car11* mRNA in mouse tissues showed that the transcript is predominantly present in the central nervous system (Aspatwar et al. 2010).

Expansion mutations of CAG repeats in ataxin-3 are responsible for spinocerebellar ataxia/Machado-Joseph disease (SCA3/MJD), a neurodegenerative disease (Kawaguchi et al. 1994). Studies using neuroblastoma cells expressing mutant ataxin-3 showed an upregulation of *CA11* similar to that of *CA8* (Hsieh 2013). Further studies on the cellular distribution of CARP XI in cultured neuronal cells from the brain tissues of humans and mice with SCA3 showed altered localization of CARP XI compared to normal specimens (Hsieh 2013). These findings suggest that the altered localization of CARP XI in SCA3 may play a role in the progression of the disease and dysfunction of the nervous system.

5.11 Concluding Remarks

CAs are widely expressed in different cell types of the central nervous system. For decades, they have been important drug targets to treat certain neurological disorders, such as epilepsy, acute mountain sickness, pseudotumor cerebri, and brain edema. In several cases, the drug designers were not initially aiming to develop CA inhibitors, and it was only later that some of them were identified as efficient CA inhibitors.

The presence of highly conserved CARPs in the brain of various species suggests important roles for these proteins in brain development and/or neural functions. Although their exact functions in the developing brain remain uncertain, the findings suggest certain roles for CARPs in the early development or differentiation of neuroprogenitor cells. Screening of patients with unknown neurological disorders for variations in CARPs may reveal novel insights into these proteins. Future research on these inactive CA isoforms may provide us with information on how these proteins work, possibly by coordination with other proteins through protein–protein interactions and might also help us to design novel treatment strategies for the diseases caused by defects or abnormal regulation of these proteins.

We still believe in the same view stated by professor Robert E. Forster 30 years ago at the Carbonic Anhydrase Conference in Spoleto, Italy: “With so many questions about its functions, some about new problems and some about old problems freshly seen, research on CA has a bright future.”

Acknowledgements Our original research has been supported by grants from the Academy of Finland, Sigrid Juselius Foundation, Jane & Aatos Erkko Foundation, and Finnish Cultural Foundation.

References

Aggarwal M, Kondeti B, McKenna R (2013) Anticonvulsant/antiepileptic carbonic anhydrase inhibitors: a patent review. Expert Opin Ther Pat 23(6):717–724

Anderson RE, Engstrom FL, Woodbury DM (1984) Localization of carbonic anhydrase in the cerebrum and cerebellum of normal and audiogenic seizure mice. Ann N Y Acad Sci 429:502–504

Ashby W (1943) Carbonic anhydrase in mammalian tissue. J Biol Chem 151:521–527

Aspatwar A et al (2013) Abnormal cerebellar development and ataxia in CARP VIII morphant zebrafish. Hum Mol Genet 22(3):417–432

Aspatwar A et al (2014) Carbonic anhydrase related proteins: molecular biology and evolution. Subcell Biochem 75:135–156

Aspatwar A et al (2015) Inactivation of ca10a and ca10b genes leads to abnormal embryonic development and alters movement pattern in zebrafish. PLoS One 10(7):e0134263

Aspatwar A, Tolvanen ME, Parkkila S (2010) Phylogeny and expression of carbonic anhydrase-related proteins. BMC Mol Biol 11:25

Aspatwar A, Tolvanen ME, Parkkila S (2013) An update on carbonic anhydrase-related proteins VIII, X and XI. J Enzyme Inhib Med Chem

Atkinson JR, Ward AA Jr (1958) Effect of diamox on intracranial pressure and blood volume. Neurology 8(1):45–50

Barnea G et al (1993) Identification of a carbonic anhydrase-like domain in the extracellular region of RPTP gamma defines a new subfamily of receptor tyrosine phosphatases. Mol Cell Biol 13(3):1497–1506

Ben-Menachem E et al (2003) Predictors of weight loss in adults with topiramate-treated epilepsy. Obes Res 11(4):556–562

Bourke RS, Kimelberg HG (1975) The effect of HCO-3 on the swelling and ion uptake of monkey cerebral cortex under conditions of raised extracellular potassium. J Neurochem 25(3):323–328

Bray GA et al (2003) A 6-month randomized, placebo-controlled, dose-ranging trial of topiramate for weight loss in obesity. Obes Res 11(6):722–733

Brown PD et al (2004) Molecular mechanisms of cerebrospinal fluid production. Neuroscience 129(4):957–970

Bruno E et al (2017) Zonisamide for essential tremor. Cochrane Database Syst Rev 8:CD009684

Cammer W (1991) Immunostaining of carbamoylphosphate synthase II and fatty acid synthase in glial cells in rat, mouse, and hamster brains suggests roles for carbonic anhydrase in biosynthetic processes. Neurosci Lett 129(2):247–250

Cammer W, Tansey FA (1988a) The astrocyte as a locus of carbonic anhydrase in the brains of normal and dysmyelinating mutant mice. J Comp Neurol 275(1):65–75

Cammer W, Tansey FA (1988b) Carbonic anhydrase immunostaining in astrocytes in the rat cerebral cortex. J Neurochem 50(1):319–322

Cammer W, Zhang H (1992) Carbonic anhydrase in distinct precursors of astrocytes and oligodendrocytes in the forebrains of neonatal and young rats. Brain Res Dev Brain Res 67(2):257–263

Celebisoy N et al (2007) Treatment of idiopathic intracranial hypertension: topiramate vs acetazolamide, an open-label study. Acta Neurol Scand 116(5):322–327

Cerminara C et al (2006) Hypohidrosis during topiramate treatment: a rare and reversible side effect. Pediatr Neurol 34(5):392–394

Chen X et al (2008) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. J Neurosci 28(48):12713–12724

Cheshire WP, Fealey RD (2008) Drug-induced hyperhidrosis and hypohidrosis: incidence, prevention and management. Drug Saf 31(2):109–126

Ghandour MS et al (1979) Double labeling immunohistochemical technique provides evidence of the specificity of glial cell markers. J Histochem Cytochem 27(12):1634–1637

Ghandour MS et al (1980) Immunochemical and immunohistochemical study of carbonic anhydrase II in adult rat cerebellum: a marker for oligodendrocytes. Neuroscience 5(3):559–571

Ghandour MS et al (1992) Carbonic anhydrase IV on brain capillary endothelial cells: a marker associated with the blood-brain barrier. Proc Natl Acad Sci U S A 89(15):6823–6827
Ghandour MS et al (2000) Mitochondrial carbonic anhydrase in the nervous system: expression in neuronal and glial cells. J Neurochem 75(5):2212–2220
Ghandour MS, Skoff RP (1991) Double-labeling in situ hybridization analysis of mRNAs for carbonic anhydrase II and myelin basic protein: expression in developing cultured glial cells. Glia 4(1):1–10
Groat JJ, Neumiller JJ (2013) Review of the treatment and management of hydrocephalus. US Pharm 38(3):HS8–HS11
Hainque E et al (2016) A randomized, controlled, double-blind, crossover trial of zonisamide in myoclonus-dystonia. Neurology 86(18):1729–1735
Halmi P, Parkkila S, Honkaniemi J (2006) Expression of carbonic anhydrases II, IV, VII, VIII and XII in rat brain after kainic acid induced status epilepticus. Neurochem Int 48(1):24–30
Hamed SA (2017) The effect of antiepileptic drugs on the kidney function and structure. Expert Rev Clin Pharmacol 10(9):993–1006
Hirota J et al (2003) Carbonic anhydrase-related protein is a novel binding protein for inositol 1,4,5-trisphosphate receptor type 1. Biochem J 372(Pt 2):435–441
Hishimoto A et al (2019) Neurexin 3 transmembrane and soluble isoform expression and splicing haplotype are associated with neuron inflammasome and Alzheimer's disease. Alzheimers Res Ther 11(1):28
Hsieh M et al (2012) Altered expression of carbonic anhydrase-related protein XI in neuronal cells expressing mutant ataxin-3. Cerebellum
Hsieh M et al (2013) Altered expression of carbonic anhydrase-related protein XI in neuronal cells expressing mutant ataxin-3. Cerebellum 12(3):338–349
Huber VJ et al (2007) Identification of arylsulfonamides as Aquaporin 4 inhibitors. Bioorg Med Chem Lett 17(5):1270–1273
Humphrey NK, Mondalas S (2018) https://www.youtube.com/watch?v=6GlNQzjii1c
Ishikawa N et al (2019) High incidence of renal stones in severely disabled children with epilepsy treated with topiramate. Neuropediatrics 50(3):160–163
Ivanov S et al (2001) Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. Am J Pathol 158(3):905–919
Jeffrey M, Wells GA, Bridges AW (1991) Carbonic anhydrase II expression in fibrous astrocytes of the sheep. J Comp Pathol 104(4):337–343
Jiao Y et al (2005) Carbonic anhydrase-related protein VIII deficiency is associated with a distinctive lifelong gait disorder in waddles mice. Genetics 171(3):1239–1246
Kadian R, Kumar A (2019) Zonisamide, in StatPearls. Treasure Island (FL)
Kallio H et al (2006) Expression of carbonic anhydrases IX and XII during mouse embryonic development. BMC Dev Biol 6:22
Kato K (1990) Sequence of a novel carbonic anhydrase-related polypeptide and its exclusive presence in Purkinje cells. FEBS Lett 271(1–2):137–140
Kawaguchi Y et al (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8(3):221–228
Kaya N et al (2011) Phenotypical spectrum of cerebellar ataxia associated with a novel mutation in the CA8 gene, encoding carbonic anhydrase (CA) VIII. Am J Med Genet B Neuropsychiatr Genet 156(7):826–834
Kellinghaus C (2009) Lacosamide as treatment for partial epilepsy: mechanisms of action, pharmacology, effects, and safety. Ther Clin Risk Manag 5:757–766
Kida E et al (2006) Carbonic anhydrase II in the developing and adult human brain. J Neuropathol Exp Neurol 65(7):664–674
Kimelberg HK, Stieg PE, Mazurkiewicz JE (1982) Immunocytochemical and biochemical analysis of carbonic anhydrase in primary astrocyte cultures from rat brain. J Neurochem 39(3):734–742
Kleiderlein JJ et al (1998) CCG repeats in cDNAs from human brain. Hum Genet 103(6):666–673

Korhonen LK, Naeaetaenen E, Hyyppae M (1964) A histochemical study of carbonic anhydrase in some parts of the mouse brain. Acta Histochem 18:336–347
Kumpulainen T et al (1983) Immunolabeling of carbonic anhydrase isoenzyme C and glial fibrillary acidic protein in paraffin-embedded tissue sections of human brain and retina. J Histochem Cytochem 31(7):879–886
Kumpulainen T et al (1985) A single-step solid phase radioimmunoassay for quantifying human carbonic anhydrase I and II in cerebrospinal fluid. Clin Chim Acta 150(3):205–212
Kumpulainen T, Korhonen LK (1982) Immunohistochemical localization of carbonic anhydrase isoenzyme C in the central and peripheral nervous system of the mouse. J Histochem Cytochem 30(4):283–292
Kwok CS, Johnson EL, Krauss GL (2017) Comparing safety and efficacy of "third-generation" antiepileptic drugs: long-term extension and post-marketing treatment. CNS Drugs 31(11):959–974
Lakkis MM et al (1997) Expression of the acatalytic carbonic anhydrase VIII gene, Car8, during mouse embryonic development. Histochem J 29(2):135–141
Lakkis MM, O'Shea KS, Tashian RE (1997) Differential expression of the carbonic anhydrase genes for CA VII (Car7) and CA-RP VIII (Car8) in mouse brain. J Histochem Cytochem 45(5):657–662
Lamont MG, Weber JT (2015) Mice deficient in carbonic anhydrase type 8 exhibit motor dysfunctions and abnormal calcium dynamics in the somatic region of cerebellar granule cells. Behav Brain Res 286:11–16
Langley OK et al (1980) Carbonic anhydrase: an ultrastructural study in rat cerebellum. Histochem J 12(4):473–483
Lehtonen J et al (2004) Characterization of CA XIII, a novel member of the carbonic anhydrase isozyme family. J Biol Chem 279(4):2719–2727
Levy JB et al (1993) The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. J Biol Chem 268(14):10573–10581
Lim LL et al (2001) Acetazolamide in women with catamenial epilepsy. Epilepsia 42(6):746–749
Luks AM et al (2010) Wilderness Medical Society consensus guidelines for the prevention and treatment of acute altitude illness. Wilderness Environ Med 21(2):146–155
Makani S et al (2012) NMDA receptor-dependent afterdepolarizations are curtailed by carbonic anhydrase 14: regulation of a short-term postsynaptic potentiation. J Neurosci 32(47):16754–16762
Maren TH (1967) Carbonic anhydrase: chemistry, physiology, and inhibition. Physiol Rev 47(4):595–781
Maryanoff BE et al (1987) Anticonvulsant O-alkyl sulfamates. 2,3:4,5-Bis-O-(1-methylethylidene)-beta-D-fructopyranose sulfamate and related compounds. J Med Chem 30(5):880–887
Masereel B et al (2002) Carbonic anhydrase inhibitors: anticonvulsant sulfonamides incorporating valproyl and other lipophilic moieties. J Med Chem 45(2):312–320
Navis A, Harden C (2016) A treatment approach to catamenial epilepsy. Curr Treat Options Neurol 18(7):30
Nishimori I et al (2013) Restoring catalytic activity to the human carbonic anhydrase (CA) related proteins VIII, X and XI affords isoforms with high catalytic efficiency and susceptibility to anion inhibition. Bioorg Med Chem Lett 23(1):256–260
Nogradi A (1993) Differential expression of carbonic anhydrase isozymes in microglial cell types. Glia 8(2):133–142
Nogradi A, Kelly C, Carter ND (1993) Localization of acetazolamide-resistant carbonic anhydrase III in human and rat choroid plexus by immunocytochemistry and in situ hybridisation. Neurosci Lett 151(2):162–165
Okamoto N et al (2001) cDNA sequence of human carbonic anhydrase-related protein, CA-RP X: mRNA expressions of CA-RP X and XI in human brain. Biochim Biophys Acta 1518(3):311–316
Ortutay C et al (eds) (2010) An evolutionary analysis of insect carbonic anhydrases. In: Berhardt LV (ed) Advances in medicine and biology, vol 7, Nova Science Publishers Inc., Hauppauge, NY, pp 145–168

Pan PW et al (2012) Brain phenotype of carbonic anhydrase IX-deficient mice. Transgenic Res 21(1):163–176
Parkkila AK et al (1997) Carbonic anhydrase II in the cerebrospinal fluid: its value as a disease marker. Eur J Clin Invest 27(5):392–397
Parkkila S et al (2001) Expression of membrane-associated carbonic anhydrase XIV on neurons and axons in mouse and human brain. Proc Natl Acad Sci U S A 98(4):1918–1923
Plog BA, Nedergaard M (2018) The glymphatic system in central nervous system health and disease: past, present, and future. Annu Rev Pathol 13:379–394
Ridderstrale Y, Hanson M (1985) Histochemical study of the distribution of carbonic anhydrase in the cat brain. Acta Physiol Scand 124(4):557–564
Roussel G et al (1979) Demonstration of a specific localization of carbonic anhydrase C in the glial cells of rat CNS by an immunohistochemical method. Brain Res 160(1):47–55
Ruusuvuori E et al (2004) Carbonic anhydrase isoform VII acts as a molecular switch in the development of synchronous gamma-frequency firing of hippocampal CA1 pyramidal cells. J Neurosci 24(11):2699–2707
Shah GN et al (2000) Mitochondrial carbonic anhydrase CA VB: differences in tissue distribution and pattern of evolution from those of CA VA suggest distinct physiological roles. Proc Natl Acad Sci U S A 97(4):1677–1682
Shah GN et al (2005) Carbonic anhydrase IV and XIV knockout mice: roles of the respective carbonic anhydrases in buffering the extracellular space in brain. Proc Natl Acad Sci U S A 102(46):16771–16776
Shimobayashi E, Wagner W, Kapfhammer JP (2016) carbonic anhydrase 8 expression in Purkinje cells is controlled by PKC gamma activity and regulates Purkinje cell dendritic growth. Mol Neurobiol 53(8):5149–5160
Smedley T, Grocott MP (2013) Acute high-altitude illness: a clinically orientated review. Br J Pain 7(2):85–94
Snyder DS et al (1983) Carbonic anhydrase, 5'-nucleotidase, and 2',3'-cyclic nucleotide-3'-phosphodiesterase activities in oligodendrocytes, astrocytes, and neurons isolated from the brains of developing rats. J Neurochem 40(1):120–127
Sterky FH et al (2017) Carbonic anhydrase-related protein CA10 is an evolutionarily conserved pan-neurexin ligand. Proc Natl Acad Sci U S A 114(7):e1253–e1262
Sturdivant NM et al (2016) Acetazolamide mitigates astrocyte cellular edema following mild traumatic brain injury. Sci Rep 6:33330
Supuran CT (2015) Acetazolamide for the treatment of idiopathic intracranial hypertension. Expert Rev Neurother 15(8):851–856
Supuran CT (2018) Applications of carbonic anhydrases inhibitors in renal and central nervous system diseases. Expert Opin Ther Pat 28(10):713–721
Svichar N et al (2006) Functional demonstration of surface carbonic anhydrase IV activity on rat astrocytes. Glia 53(3):241–247
Svichar N et al (2009) Carbonic anhydrases CA4 and CA14 both enhance AE3-mediated Cl–HCO3-exchange in hippocampal neurons. J Neurosci 29(10):3252–3258
Swenson ER (2014) Carbonic anhydrase inhibitors and high altitude illnesses. Subcell Biochem 75:361–386
Szczygielski J et al (2019) Brain edema formation and functional outcome after surgical decompression in murine closed head injury are modulated by acetazolamide administration. Front Neurol 10:273
Tanimoto T et al (2005) Immunohistochemical co-expression of carbonic anhydrase II with Kv1.4 and TRPV1 in rat small-diameter trigeminal ganglion neurons. Brain Res 1044(2): 262–265
Taniuchi K et al (2002a) cDNA cloning and developmental expression of murine carbonic anhydrase-related proteins VIII, X, and XI. Brain Res Mol Brain Res 109(1–2):207–215
Taniuchi K et al (2002b) Developmental expression of carbonic anhydrase-related proteins VIII, X, and XI in the human brain. Neuroscience 112(1):93–99

Temperini C et al (2010) The coumarin-binding site in carbonic anhydrase accommodates structurally diverse inhibitors: the antiepileptic lacosamide as an example and lead molecule for novel classes of carbonic anhydrase inhibitors. J Med Chem 53(2):850–854
Thurtell MJ, Wall M (2013) Idiopathic intracranial hypertension (pseudotumor cerebri): recognition, treatment, and ongoing management. Curr Treat Options Neurol 15(1):1–12
Tolvanen ME et al (2012) Analysis of evolution of carbonic anhydrases IV and XV reveals a rich history of gene duplications and a new group of isozymes. Bioorg Med Chem
Tschirgi RD, Frost RW, Taylor JL (1954) Inhibition of cerebrospinal fluid formation by a carbonic anhydrase inhibitor, 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (diamox). Proc Soc Exp Biol Med 87(2):373–376
Turkmen S et al (2006) Cerebellar hypoplasia and quadrupedal locomotion in humans as a recessive trait mapping to chromosome 17p. J Med Genet 43(5):461–464
Turkmen S et al (2009) CA8 mutations cause a novel syndrome characterized by ataxia and mild mental retardation with predisposition to quadrupedal gait. PLoS Genet 5(5):e1000487
Vujovic M et al (2015) Thyroid hormone drives the expression of mouse carbonic anhydrase Car4 in kidney, lung and brain. Mol Cell Endocrinol 416:19–26
Wall M et al (2014) The idiopathic intracranial hypertension treatment trial: clinical profile at baseline. JAMA Neurol 71(6):693–701
Wall M, McDermott MP, Kieburtz KD, Corbett JJ, Feldon SE, Friedman DI, Committee et al (2014) Effect of acetazolamide on visual function in patients with idiopathic intracranial hypertension and mild visual loss: the idiopathic intracranial hypertension treatment trial. JAMA 311(16)1641–1651
Whitelaw A, Kennedy CR, Brion (2001) Diuretic therapy for newborn infants with posthemorrhagic ventricular dilatation. Cochrane Database Syst Rev 2001(2):CD002270
Yan J et al (2007) Effects of carbonic anhydrase VIII deficiency on cerebellar gene expression profiles in the wdl mouse. Neurosci Lett 413(3):196–201
Zhuang GZ et al (2015) Carbonic anhydrase-8 regulates inflammatory pain by inhibiting the ITPR1-cytosolic free calcium pathway. PLoS One 10(3):e0118273