SYMPOSIUM REPORT

Two developmental switches in GABAergic signalling: the K^{+}–Cl^{-} cotransporter KCC2 and carbonic anhydrase CAVII

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GABAergic signalling has the unique property of 'ionic plasticity', which is based on short-term and long-term changes in the Cl^{-} and HCO_3^{-} ion concentrations in the postsynaptic neurones. While short-term ionic plasticity is caused by activity-dependent, channel-mediated anion shifts, long-term ionic plasticity depends on changes in the expression patterns and kinetic regulation of molecules involved in anion homeostasis. During development the efficacy and also the qualitative nature (depolarization/excitation *versus* hyperpolarization/inhibition) of GABAergic transmission is influenced by the neuronal expression of two key molecules: the chloride-extruding K^{+}–Cl^{-} cotransporter KCC2, and the cytosolic carbonic anhydrase (CA) isoform CAVII. In rat hippocampal pyramidal neurones, a steep up-regulation of KCC2 accounts for the 'developmental switch', which converts depolarizing and excitatory GABA responses of immature neurones to classical hyperpolarizing inhibition by the end of the second postnatal week. The immature hippocampus generates large-scale network activity, which is abolished in parallel by the up-regulation of KCC2 and the consequent increase in the efficacy of neuronal Cl^{-} extrusion. At around postnatal day 12 (P12), an abrupt, steep increase in intrapyramidal CAVII expression takes place, promoting excitatory responses evoked by intense GABAergic activity. This is largely caused by a GABAergic potassium transient resulting in spatially widespread neuronal depolarization and synchronous spike discharges. These facts point to CAVII as a putative target of CA inhibitors that are used as antiepileptic drugs. KCC2 expression in adult rat neurones is down-regulated following epileptiform activity and/or neuronal damage by BDNF/TrkB signalling. The lifetime of membrane-associated KCC2 is very short, in the range of tens of minutes, which makes KCC2 ideally suited for mediating GABAergic ionic plasticity. In addition, factors influencing the trafficking and kinetic modulation of KCC2 as well as activation/deactivation of CAVII are obvious candidates in the ionic modulation of GABAergic responses. The down-regulation of KCC2 under pathophysiological conditions (epilepsy, damage) in mature neurones seems to reflect a 'recapitulation' of early developmental mechanisms, which may be a prerequisite for the re-establishment of connectivity in damaged brain tissue.

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Introduction

A characteristic feature of all structures in the developing central nervous system, including the spinal cord, sensory systems, brain stem as well as the cortex, is the presence of endogenous large-scale spontaneous activity (Feller, 1999; Penn & Shatz, 1999; Zhang & Poo, 2001; Ben-Ari, 2002). The temporal patterns as well as cellular and network mechanisms related to early network events seem to exhibit considerable variations among distinct neuronal networks as well as during the ontogeny of a given structure. Nevertheless, it is generally believed that this type of endogenous activity has an important role in the activity-dependent wiring of neuronal circuits,

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and that the maturation of GABAergic inhibition is a crucial factor during later developmental stages when the large-scale endogenous events disappear (Garaschuk *et al.* 2000; Ben-Ari, 2001; Owens & Kriegstein, 2002; Kandler, 2004; Sernagor *et al.* 2003).

The aim of the present review is to summarize recent data and conclusions related to the role of gamma-aminobutyric acid (GABA) in early network activity of mammalian cortical structures. Most of this work has been carried out on the rodent hippocampus, where the developmental change in the action of GABA from a depolarizing (and often excitatory) transmitter in immature neurones to a hyperpolarizing and typically inhibitory one has gained an enormous amount of attention. The key mechanisms in such a change in transmitter function must be postsynaptic. In the case of GABA and its sister transmitter glycine, these mechanisms are based on the maturation of neuronal Cl^- homeostasis, which produces a negative shift in the equilibrium potential of Cl^- during neuronal development and differentiation (Mueller *et al.* 1984; Ben-Ari *et al.* 1989; Luhmann & Prince, 1991; Zhang *et al.* 1991). This early 'ontogenetic switch' in ionotropic GABAergic transmission is attributable to the developmental expression of the neurone-specific K^+–Cl^- cotransporter, KCC2 (Fig. 1*A*; Rivera *et al.* 1999; Hubner *et al.* 2001). In addition to this, there is another molecular switch, the neuronal expression of the carbonic anhydrase isoform VII (CAVII), which can transiently make GABAergic transmission functionally excitatory in mature neurones, especially under conditions of massive activation of $GABA_A$ receptors (Ruusuvuori *et al.* 2004).

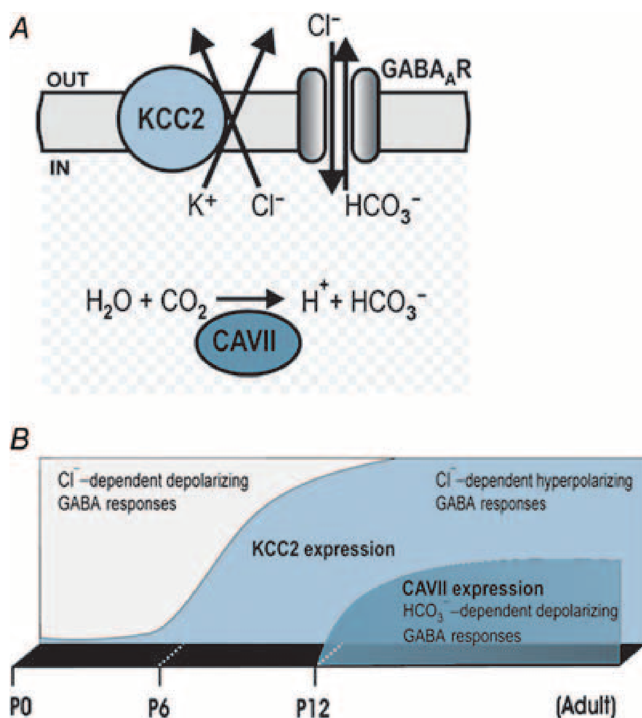

Figure 1. KCC2 and CAVII as developmental switches in GABAergic transmission
A, the current across $GABA_A$ receptors ($GABA_AR$) is carried by Cl^- and HCO_3^-. While the Cl^--mediated current can be depolarizing or hyperpolarizing, depending on the developmental expression of KCC2, the HCO_3^- current is depolarizing in all neurones irrespective of developmental stage. In order to have a significant effect, the HCO_3^- current has to be supported by the conversion of CO_2 into HCO_3^- (and protons) by an intraneuronal carbonic anhydrase, such as the isoform CAVII that is present in cortical principal neurones (see text for further details). *B*, a scheme showing the developmental patterns of expression of KCC2 and CAVII in rat hippocampal pyramidal neurones. The first main ontogenetic switch from depolarizing to hyperpolarizing $GABA_A$ action is caused by the developmental expression of KCC2 that shows a steep increase during the first two postnatal weeks. A sharp increase in the expression of CAVII that takes place at around P12 promotes transient excitatory GABAergic responses which are caused by the $GABA_A$-mediated HCO_3^- current and, in brain tissue, by the consequent increase in interstitial K^+ (see text for detailed explanation).

Neuronal chloride homeostasis

Adult mammalian central neurones, with some exceptions (Gulacsi *et al.* 2003; Bartho *et al.* 2004), are atypical cells in that they maintain a low intracellular Cl^- concentration which is, of course, a prerequisite for 'classical' hyperpolarizing inhibition mediated by ionotropic GABA and glycine receptors (Kaila, 1994). The evolutionary trade-off for this mode of postsynaptic signalling must have included, among other consequences, a compromised capacity for (and/or a requirement for novel mechanistic designs for) the control of intracellular pH and cellular volume. In most cells, both of these homeostatic functions are largely dependent on a large source of intracellular Cl^- that is usually required for the uptake of HCO_3^- (in the case of pH regulation) or for net efflux of K^+ in response to cell swelling (Alvarez-Leefmans & Russell, 1990; Kaila & Ransom, 1998). Hence, the high intracellular Cl^- of immature neurones can be considered the rule, rather than the exception, in comparative cellular physiology.

Cl^- homeostasis in brain cells is mainly controlled by cation–chloride cotransporters (CCCs), which mediate electrically neutral Cl^- uptake fuelled by Na^+ (the Na^+–K^+–2 Cl^- cotransporter, NKCC1) or Cl^- extrusion fuelled by K^+ (the K^+–Cl^- cotransporters, KCC1–4) (Hiki *et al.* 1999; Race *et al.* 1999; Delpire & Mount, 2002; Payne *et al.* 2003; Mercado *et al.* 2004). These secondary active transporters do not directly consume ATP, but they derive their energy from the Na^+ and K^+ gradients generated by the Na^+,K^+-ATPase. In addition to the CCCs, the Na^+-dependent and Na^+-independent anion exchangers (NDAE and AE, respectively) have an influence on neuronal Cl^- since they mediate an exchange of Cl^- for HCO_3^-. For the present purposes, we will focus on the expression patterns and functions of one member of the KCCs and of NKCCs: these are KCC2 and NKCC1.

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It is widely believed that uptake of Cl^- in immature neurones is mediated by NKCC1 (Payne *et al.* 2003; Yamada *et al.* 2004). Interestingly, although $GABA_A$-mediated depolarization is not present in NKCC1 knock-out mice (Sung *et al.* 2000), no significant phenotype has been observed in the central nervous system.

Unfortunately, there are no drugs available that would act as specific inhibitors of K^+–Cl^- cotransport (not to mention an isoform-specific inhibitor!). Two compounds, furosemide and DIOA (R(+)-[(2-n-butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1H-inden-5-yl)oxy] acetic acid), are widely used, but they have effects on a wide variety of enzymes and receptors, which means that careful control experiments must be run to confirm that they are working in a manner that is consistent with any given experimental strategy. In particular, it should be emphasized that both of these drugs are more potent blockers of NKCC1 than of KCC2 (Payne, 1997), and they also inhibit carbonic anhydrases (Supuran *et al.* 2003; and unpublished observations by K. Kaila, J. Kolehmainen, C.T. Supuran & J. Voipio). Another complication that is often ignored in electrophysiological experiments is that Cs^+, a widely used internal cation in whole-cell patch clamp experiments, is a very poor substrate for KCC2, resulting in a block of Cl^- extrusion (Kakazu *et al.* 2000; Williams & Payne, 2004). Unfortunately, the kinetic data in the only published model of neuronal K^+–Cl^- cotransport in neurones (Staley & Proctor, 1999) were obtained using Cs^+ in whole-cell experiments – and hence, in the absence of functional KCC2!

Developmental up-regulation of KCC2

Pioneering work (Deisz & Lux, 1982; Misgeld *et al.* 1986; Thompson *et al.* 1988*a*,*b*; Thompson & Gähwiler, 1989; Jarolimek *et al.* 1999) showed that neuronal Cl^- extrusion is largely attributable to K^+–Cl^- cotransport, and that there was a developmentally regulated increase in the efficacy of Cl^- extrusion during neuronal maturation (Luhmann & Prince, 1991; Zhang *et al.* 1991). The molecular identity of the extrusion mechanism became clear in studies on the expression patterns of KCC2 in the immature rat hippocampus (Fig. 2*A*). The well-known negative shift in the reversal potential of ionotropic GABA action (E_{GABA}) was paralleled by the up-regulation of this molecule as seen in *in situ* hybridization (Rivera *et al.* 1999). (A similar result was published by Lu *et al.* (1999) but, surprisingly, these workers also found KCC2 expression in dorsal root ganglion neurones, which casts doubts on the specificity of their assays.) In addition, KCC2 shows a brain-specific expression pattern (Payne *et al.* 1996) and

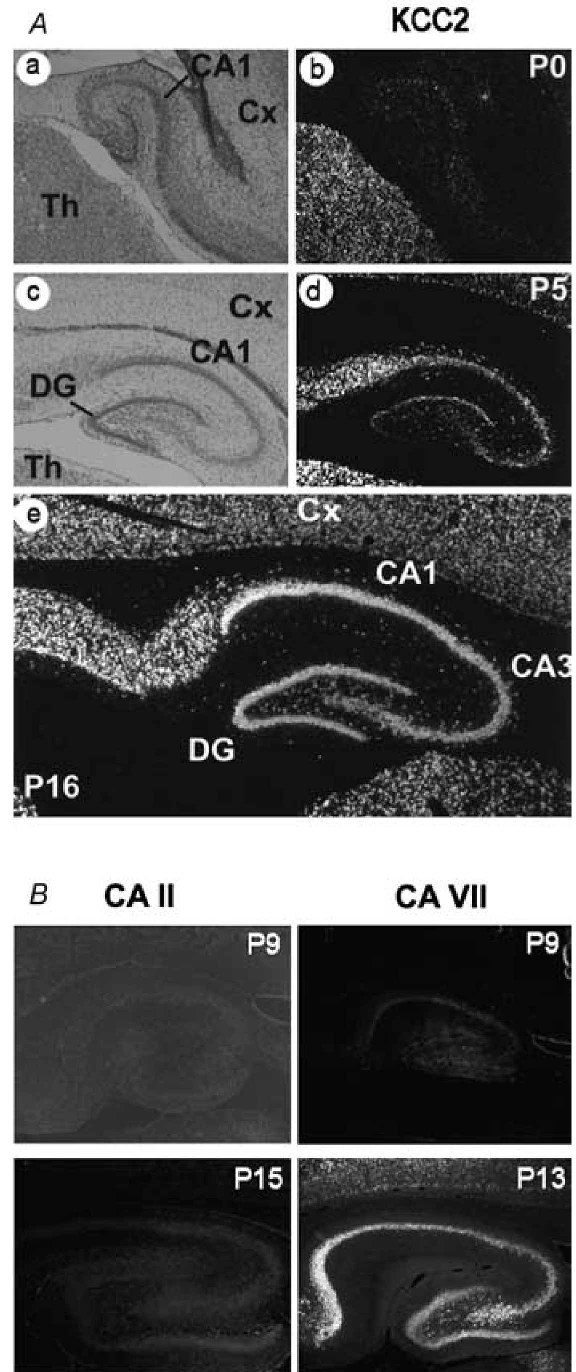

Figure 2. Developmental patterns of up-regulation of KCC2 and CAVII
A, radioactive *in situ* hybridization (panels *b*, *d* and *e*) showing steep increase in KCC2 mRNA by the end of the second postnatal week in rat hippocampal principal neurones (eosin haematoxylin counter staining, panels *a* and *c*; Th, thalamus; Cx, neocortex; DG, dentate gyrus). *B*, fluorescent *in situ* hybridizations indicating a pronounced increase in the expression of CAVII mRNA at around P12. Note the absence of a marked change in the level of the CAII transcript. (*A* is modified from Rivera *et al.* 1999 (©Nature Publishing Group (1999); http://www.nature.com/), *B* from Ruusuvuori *et al.* 2004 (©2004 by the Society for Neuroscience)).

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the neurone specificity was further confirmed in subsequent work (Rivera *et al.* 1999; Williams *et al.* 1999; DeFazio *et al.* 2000; Hubner *et al.* 2001; Stein *et al.* 2004). Obviously, a correlation between the developmental expression patterns of KCC2 and the negative shift of E_{GABA} does not demonstrate a causal relationship. Direct evidence for this was obtained using gene knock-down of KCC2 in slice cultures based on the use of antisense oligonucleotides. This resulted in a block of KCC2 expression and to near abolition of the negative (hyperpolarizing) driving force of the $GABA_A$-mediated current (Rivera *et al.* 1999). It is likely that NDAE is capable of mediating Cl^- extrusion in neurones devoid of KCC2 (see also Kaila, 1994; Gulacsi *et al.* 2003), which explains the small residual hyperpolarizing GABAergic action. A full KCC2 'knock-out' is lethal, and mice with such a gene disruption die at birth due to the expected malfunction of the respiratory centre (Hubner *et al.* 2001).

The developmental patterns of KCC2 expression suggest that it is a molecule that is a very useful indicator of the state of neuronal maturation (see also Shimizu-Okabe *et al.* 2002; Ikeda *et al.* 2003; Payne *et al.* 2003). Here, an interesting finding is that a minority of central neurones do not express KCC2. These include the dopaminergic neurones in the striatum (Gulacsi *et al.* 2003) as well as a population of neurones in the nucleus reticularis thalami (Bartho *et al.* 2004). With regard to the dopaminergic neurones, one might postulate that the weak GABAergic inhibition they experience is important for tonic spiking and the consequent secretion of dopamine. Perhaps a lack of KCC2 is also a distinct feature of other neurones that secrete neuromodulatory amines – a question that needs attention in future work.

In the developing rat hippocampus, the increase in the efficacy of Cl^- extrusion and expression of KCC2 take place by the end of the second postnatal week (Zhang *et al.* 1991; Rivera *et al.* 1999). However, one should be careful in comparisons across species, keeping in mind that the time of birth provides no solid point of reference. For instance, the rat is born at a very immature state which makes early postnatal pups useful model organisms in studies of human cortical development, corresponding roughly to the last trimester of pregnancy (cf. Clancy *et al.* 2001; Avishai-Eliner *et al.* 2002; Erecinska *et al.* 2004). Furthermore, the developmental time course of KCC2 expression is neurone-type specific within a given species as seen in the earlier expression of this transporter in, e.g. thalamus than in the cortex (Rivera *et al.* 1999; Li *et al.* 2002; Wang *et al.* 2002). It is also intriguing that KCC2 is highly expressed in dendritic spines (Gulyas *et al.* 2001), which are major postsynaptic targets of glutamatergic rather than GABAergic inputs (Freund & Buzsaki, 1996). This raises the question whether KCC2 has functions in neuronal signalling which are not directly related to the maintenance of the postsynaptic Cl^- gradient underlying 'classical' hyperpolarizing inhibition (cf. Kaila, 1994).

What triggers the expression of KCC2 during neuronal development? In a recent paper based on hippocampal cultures, Ganguly *et al.* (2001) suggested that GABA itself is the signal that activates the intracellular cascades controlling KCC2 gene expression. This hypothesis, where the depolarizing action of GABA is shut off in a feed-back manner is an elegant one, but subsequent work has shown that the increase in KCC2 protein expression (Ludwig *et al.* 2003) as well as the negative shift in E_{GABA} (Titz *et al.* 2003) can take place in cultures in the constant presence of $GABA_A$ antagonists. Factors acting on the tyrosine kinase receptor such as insulin-like growth factor and BDNF have been shown to regulate the expression and function of KCC2 during development (Kelsch *et al.* 2001; Aguado *et al.* 2003). In addition, recent work comparing the quantitative expression of KCC2 protein and the efficacy (functionality) of KCC2 in native hippocampal tissue *versus* cultures suggests the absence of some critical factor(s) in cultures that are needed for the KCC2 transporter to become functional (unpublished observations of S. Khirug, K. Huttu, A. Ludwig, S. Smirnov, J. Voipio, C. Rivera, K. Kaila & L. Khiroug).

Early spontaneous activity and KCC2

In a highly cited paper published by Ben-Ari and coworkers (Ben-Ari *et al.* 1989), the shift from depolarizing to hyperpolarizing GABA action in the rat hippocampus was found to take place in parallel with the disappearance of large-scale network events, which were termed 'giant depolarizing potentials' (GDPs) in recordings with intracellular sharp microelectrodes. The developmental time scale of these sequences of events at the cellular and network level has been subject to recent revisions (Khazipov *et al.* 2004). Nevertheless, the intriguing temporal correlation with early endogenous activity and the depolarizing action of GABA has led to the hypothesis that the generation of endogenous patterns of activity in developing networks is set by the action of depolarizing GABA and, hence, by the properties of the interneuronal network.

When examining the literature on developing networks as a whole, it appears that transmitter actions other than, or in addition to, depolarizing GABA are often involved in driving the endogenous activity that is characteristic of immature CNS structures (see Introduction). However, depolarizing GABA seems to play a role during certain developmental time windows in various brain circuits, and in cortical structures the present evidence does suggest an important role for GABA-mediated excitation (Ben-Ari, 2002; Leinekugel *et al.* 2002; Owens & Kriegstein, 2002). Nevertheless, even in the immature hippocampus it is not clear at all that GABAergic interneurones are

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responsible for the rhythmogenesis of early spontaneous activity. Recently, we have obtained strong evidence for the hypothesis that the temporal patterns of hippocampal 'GDPs' and their counterparts at the network level are shaped by glutamatergic neurones (S. Sipilä, K. Huttu, J. Voipio & K. Kaila, unpublished observations).

Neuronal damage: 'recapitulation' of KCC2 expression patterns and network activity

The fact that neuronal trauma leads to a de-differentiation of the neuronal phenotype is known from a number of studies, including those on ion transport (Nabekura *et al.* 2002; Toyoda *et al.* 2003; Payne *et al.* 2003). For instance, it is known that changes occur in the $GABA_A$ subunit composition in the dentate gyrus that resemble those during early development (Buhl *et al.* 1996).

It is a well-known fact that epileptic activity leads to an increase in the expression of brain-derived neurotrophic factor (BDNF) and its receptor TrkB (Binder *et al.* 2001; Huang & Reichardt, 2001, 2003). We were intrigued by the observation that following *in vivo* kindling, the expression of KCC2 showed a rapid, pronounced fall in those regions of the epileptic hippocampus where BNDF–TrkB up-regulation is most salient (Rivera *et al.* 2002). Further *in vitro* experiments established a direct causal link from TrkB activation to KCC2 down-regulation (Rivera *et al.* 2002, 2004). Using transgenic mice with point mutations in their TrkB receptors (see Minichiello *et al.* 1998), it was found that the down-regulation of KCC2 requires the activation of the two major TrkB-mediated signalling cascades, the PLCγ and Shc activated pathways. In contrast, activation of the Shc cascade in isolation leads, intriguingly, to an increase in KCC2 expression (Rivera *et al.* 2004). Hence, these data may shed light also on the involvement of trophic signalling in the developmental up-regulation of KCC2 (see above).

The above observations raise a number of questions and hypotheses: perhaps the fall in KCC2 is part of a wider picture where the afflicted neurones assume neonatal features that are crucial for the *de novo* targeting of neurones during damage-related rewiring? Perhaps a down-regulation of ion extrusion is an energy-saving strategy that will help the neurones to survive a time window where hyperexcitability leads to an enormous energy metabolic load. It is obvious that questions of this kind cannot be resolved just by a series of experiments – they are paradigmatic in nature, and hence require a large bulk of experimental data from future work to provide solid evidence for, or against, definitive hypotheses. However, an intriguing observation in this context is that in the chronically epileptic human hippocampus, the sclerosis of the CA1 area is associated with an enhanced subicular output. In particular, some of the principal neurones in the subiculum have depolarizing and excitatory GABA responses (Cohen *et al.* 2002, 2003). Perhaps these are indications of a 'recapitulation' of a developmental programme (Cohen *et al.* 2003; Payne *et al.* 2003), which is necessary for repair mechanisms that operate at the network level. An exciting possibility is that the interictal activity which is a characteristic feature of the epileptic brain shares some fundamental features with the early large-scale spontaneous activity that is implicated in the formation of neuronal networks.

In addition to down-regulation, KCC2 expression can be enhanced within a certain time window in response to neuronal hyperexcitability and/or trauma. Here, caution is needed in functional interpretations, since a dramatic enhancement of intrasomatic staining is seen in KCC2 immunohistochemistry (based on a well-characterized, specific antibody that binds to the cytoplasmic C-terminus of this transport protein; Ludwig *et al.* 2003) in a number of *in vivo* and *in vitro* models of neuronal hyperexcitability and damage (Lahtinen *et al.* 2002, 2004). In order to find out whether an adaptive increase in the expression of functional KCC2 takes place in response to trauma, direct measurements of the capacity of neuronal Cl^- extrusion are needed.

Carbonic anhydrase isoform VII (CAVII) and bicarbonate-dependent GABAergic excitation

Massive, synchronous excitation of hippocampal pyramidal neurones can be achieved by high-frequency stimulation (HFS) of hippocampal interneurones in the absence of ionotropic glutamatergic transmission (Kaila *et al.* 1997; Smirnov *et al.* 1999; Ruusuvuori *et al.* 2004). The ionic mechanism of this type of GABAergic excitation is strictly dependent on the presence of HCO_3^-, which is the only physiological ion in addition to Cl^- that is able to mediate a current across $GABA_A$ receptors.

The equilibrium potential of HCO_3^- (E_{HCO_3}) is at a much more positive level (around −10 mV) than that of Cl^-, since E_{HCO_3} is set by mechanisms that control intracellular pH regulation (Kaila & Voipio, 1987). Hence, bicarbonate mediates an inward, depolarizing current (Kaila & Voipio, 1987; Kaila *et al.* 1993; Gulledge & Stuart, 2003). During the $GABA_A$ receptor-mediated efflux of HCO_3^-, the intracellular level of this anion is strongly buffered by the presence of intraneuronal carbonic anhydrase (CA) (Pasternack *et al.* 1993) that uses CO_2 as a substrate for the generation of HCO_3^- (Kaila *et al.* 1990). As shown in crayfish muscle fibres (Kaila & Voipio, 1987; Kaila *et al.* 1989) as well as neurones (Voipio *et al.* 1991), the depolarization caused by the HCO_3^- current leads to an accumulation of intraneuronal Cl^- and consequently, there is a large positive shift in E_{GABA}.

Notably, in mammalian brain tissue, the HCO_3^-- and CA-dependent anion redistribution induces a profound

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increase in interstitial K^+ during prolonged GABAergic stimulation (also during $GABA_A$ agonist application in the absence of spiking; Barolet & Morris, 1991), which results in a long-lasting, non-synaptic depolarization. But why should $GABA_A$ receptor activation produce a large potassium transient? Here one should note that the only biophysically feasible way to clear the HCO_3^--dependent postsynaptic intraneuronal Cl^- load is to link the subsequent, homeostatic net efflux of Cl^- in a 1 : 1 manner to an efflux of a K^+ (Voipio & Kaila, 2000). Whether this coflux is mediated by channels and/or transporters is not known. Nevertheless, the increase in extracellular K^+ is an ionic transient that is exactly what is expected to take place in response to massive $GABA_A$ receptor activation. In accordance with this, the membrane potential of pyramidal neurones is influenced by the increase in K^+, and hence it achieves a level that is much more depolarized than the value of E_{GABA} (Kaila *et al.* 1997; Smirnov *et al.* 1999). As might be expected, membrane-permeant CA inhibitors strongly suppress HFS-induced GABAergic excitation, while impermeant CA inhibitors (such as benzolamide) have no effect (Ruusuvuori *et al.* 2004).

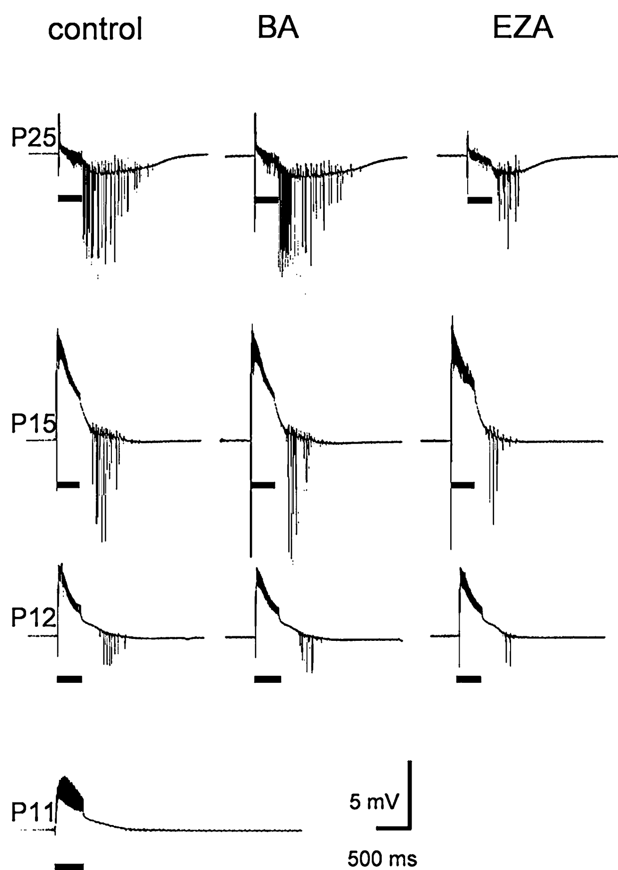

Figure 3. Ontogeny of CAVII-dependent high-frequency stimulation (HFS) induced afterdischarges as seen in field potential recordings in CA1
Note emergence of abrupt synchronous spiking evoked by HFS at P12 and the selective sensitivity to the membrane-permeant CA inhibitor ethoxyzolamide (EZA) but not to the impermeant derivative benzolamide (BA). (From Ruusuvuori *et al.* 2004, ©2004 by the Society for Neuroscience).

When examining the ontogeny of the HCO_3^--dependent GABAergic excitation, we found a sharp expression profile where HFS of GABAergic inputs does not lead to synchronous spiking in the early postnatal hippocampus (Figs 1*B* and 3; Ruusuvuori *et al.* 2004). The developmental profiles of action of CA inhibitors on HFS-induced synchronous spiking as well as fluorescence measurements of intracellular pH transients indicated that the expression of intrapyramidal CA, i.e. the ‘developmental switch’ that is necessary for HFS-induced GABAergic excitation, commences at P10–P12.

From the 14 CA isozymes that are at present known, five show a cytosolic localization (CAs I–III, CAVII and CAXIII; for references, see Supuran *et al.* 2003; Pastorekova *et al.* 2004; see also Lakkis *et al.* 1997). Using *in situ* hybridization, the intrapyramidal carbonic anhydrase was identified as CAVII (Fig. 2*B*; Ruusuvuori *et al.* 2004; see also Lakkis *et al.* 1997). Considering (1) the role of CAVII in GABAergic excitation and (2) the tight link between epileptiform actitity and interstitial K^+ transients, it is intriguing to speculate that CAVII might be a major target of those anticonvulsants that are known to be inhibitors of carbonic anhydrase (Wyllie, 1997; Masereel *et al.* 2002; Scozzafava *et al.* 2004). It is of particular interest that CAVII has an extremely high catalytic activity, rivalling the well-known high activity of CAII (Earnhardt *et al.* 1998). Obviously, CAVII is a promising target for the design of novel anticonvulsant compounds. It is also likely that the catalytic action of CAVII is modulated by a variety of compounds such as monoamines and tricarboxylic acids (Supuran *et al.* 2003), which suggests that these endogenous modulators would also shape the properties of GABAergic transmission activation/deactivation of intraneuronal CA activity.

Ionic plasticity of GABAergic inhibition

It is apparent that GABAergic signalling has the unique property of ‘ionic plasticity’ which is based on both short-term and long-term shifts in the concentrations of Cl^- and HCO_3^- in postsynaptic neurones. Short-term ionic plasticity manifests itself not only as a change in the efficacy of GABAergic inhibition, but sometimes as a change from functional inhibition to GABAergic excitation that takes place in response to massive $GABA_A$ receptor activation during high-frequency activity of interneuronal networks. This suggests that the qualitative change of action of GABAergic transmission from inhibitory to excitatory is likely to take place during, e.g. epileptiform activity as well as during standard protocols

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of LTP induction that involve bursts of tetanic stimulation. An obvious task in future work is to examine the role of CAVII in the induction of LTP as well as in epileptogenesis (see also Sun & Alkon, 2002).

While short-term ionic plasticity of GABAergic transmission is directly shaped by activity-dependent ion shifts and by the efficacy of transport mechanisms involved in the recovery thereof, long-term ionic plasticity depends on changes in the expression patterns of proteins involved in the regulation of intraneuronal anions. The present review has its focus on two of them, KCC2 and CAVII. In the case of KCC2, there appears to be an intriguing cross-talk among GABAergic transmission, KCC2 expression and neurotrophin signalling during both neuronal development and damage. The lifetime of membrane-associated KCC2 is very short, in the range of tens of minutes, which makes KCC2 ideally suited for mediating GABAergic ionic plasticity (Wardle & Poo, 2003; Rivera *et al.* 2004; Kovalchuk *et al.* 2004). In addition, factors influencing the trafficking and kinetic modulation of KCC2 as well as activation/deactivation of CAVII are obvious candidates in the ionic modulation of GABAergic responses. It will be interesting to examine whether the expression of CAVII and other molecules that shape GABAergic transmission via anion homeostasis are interconnected by common intracellular signalling cascades, especially those involving neurotrophin actions. Work along these lines is likely to shed light on the fundamental mechanisms that are involved in the wiring, miswiring and re-wiring of neuronal networks.

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