

Adenylyl Cyclases: Expression in the Developing Rat Thalamus and Their Role in Absence Epilepsy

Petra Ehling · Tatyana Kanyshkova · Arnd Baumann ·
Peter Landgraf · Sven G. Meuth ·
Hans-Christian Pape · Thomas Budde

Received: 5 March 2012 / Accepted: 30 March 2012 / Published online: 25 April 2012
© Springer Science+Business Media, LLC 2012

Abstract Adenylyl cyclases (ACs) synthesize the second messenger cyclic AMP (cAMP) which influences the function of multiple ion channels. Former studies point to a malfunction of cAMP-dependent ion channel regulation in thalamocortical relay neurons that contribute to the development of the absence epileptic phenotype of a rat genetic model (WAG/Rij). Here, we provide detailed information about the thalamic gene and protein expression of Ca^{2+} /calmodulin-activated AC isoforms in rat thalamus. Data from WAG/Rij were compared to those from non-epileptic controls (August-Copenhagen Irish rats) to elucidate whether differential expression of ACs contributes to the dysregulation of thalamocortical activity. At one postnatal stage

(P21), we found the gene expression of two specific Ca^{2+} -activated AC isoforms (AC-1 and AC-3) to be significantly down-regulated in epileptic tissue, and we identified the isoform AC-1 to be the most prominent one in both strains. However, Western blot data and analysis of enzymatic AC activity revealed no differences between the two strains. While basal AC activity was low, cAMP production was boosted by application of a forskolin derivative up to sevenfold. Despite previous hints pointing to a major contribution of ACs, the presented data show that there is no apparent causality between AC activity and the occurrence of the epileptic phenotype.

Keywords Cyclic AMP · Adenylyl cyclase ·
Thalamocortical relay neuron · Absence epilepsy

P. Ehling (✉) · S. G. Meuth
Neurology Clinic–Inflammatory Disorders of the Nervous
System and Neurooncology, and Institute of Physiology
I - Neuropathophysiology, Westfälische Wilhelms-University,
ICB, Mendelstr. 7,
48149 Muenster, Germany
e-mail: Petra.Ehling@uni-muenster.de

P. Ehling
Otto Creutzfeldt Center for Cognitive and Behavioral
Neuroscience, Westfälische Wilhelms-University,
Muenster, Germany

T. Kanyshkova · H.-C. Pape · T. Budde
Institute of Physiology I - Neurophysiology, Westfälische
Wilhelms-University,
Muenster, Germany

A. Baumann
Institute of Complex Systems–Zelluläre Biophysik (ICS-4),
Research Center Juelich GmbH,
Juelich, Germany

P. Landgraf
PG Neuralomics, Leibniz Institute for Neurobiology,
Magdeburg, Germany

Introduction

During the behavioral states of sleep and wakefulness, the thalamocortical (TC) system is characterized by two fundamentally different states of activity (Steriade 1997): low-frequency (<15 Hz) oscillatory activity prevails during natural sleep, deep anesthesia, and epileptic seizures; whereas high-frequency oscillatory activity in the gamma range and tonic activity dominate during wakefulness. On the cellular level, slow oscillatory activity is associated with rhythmic bursts of action potentials in TC neurons, while tonic activity is associated with sequences of single action potentials. The latter is thought to underlie the faithful transfer of sensory information from the periphery to the cortex (Sherman 2007). On the molecular level, TC neurons express a variety of different ion channel types, which differentially contribute to the characteristic patterns of electrical activity. Transmitters of the ascending brainstem system, e.g., noradrenaline, modulate

the activity of these ion channels and thereby network activity by stimulating β -adrenergic receptors and inducing cAMP-dependent cellular signals (McCormick and Huguenard 1992).

Adenylyl cyclases (AC) catalyze the conversion of ATP into cAMP. cAMP is a potent second messenger that can act in not only phosphorylation-dependent, i.e., protein kinase A (PKA)-mediated, but also phosphorylation-independent ways (Defer et al. 2000). In mammals, nine isoforms of membrane-bound ACs are known, each encoded by a single gene (AC-1–AC-9). AC activity is controlled by hormones and neurotransmitters acting via G protein-coupled receptors as well as by additional modulators. Beside G proteins, modulators of AC activity include Ca^{2+} -calmodulin (CaM), various kinases, and phosphatases as well as the plant-derived diterpene forskolin. Molecular biological, immunohistochemical, and biochemical techniques revealed the functional expression of CaM-stimulated AC-1 and AC-8 in the rodent thalamus (Matsuoka et al. 1997; Ihnatovych et al. 2002; Nicol et al. 2006; Visel et al. 2006; Conti et al. 2007).

In TC neurons, increments in intracellular cAMP levels induced either by applying cAMP directly or incubation with forskolin, as well as stimulation of membrane receptors, enhanced hyperpolarization-activated inward currents (I_h), increased L- and N-type Ca^{2+} currents, and reduced Ca^{2+} -activated K^+ currents (Biella et al. 2001; Meuth et al. 2002, 2006; Frère and Lüthi 2004). It has been demonstrated that the cAMP-dependent enhancement of I_h in the thalamus contributes to the control of rhythmic network activities related to sleep and epilepsy (Bal and McCormick 1996; Lüthi and McCormick 1999; Kanyshkova et al. 2009). Moreover, two independent genetic rat models of absence epilepsy (AE) revealed that abnormal regulation of I_h accompanies the pathogenesis in that there is a reduction in responsiveness to cAMP associated with a selective increase in the expression of a channel isoform (hyperpolarization-activated and cyclic nucleotide-gated cation channel-1, HCN1) that carries a cAMP-insensitive I_h in TC neurons (Budde et al. 2005; Kuisle et al. 2006; Kanyshkova et al. 2011). Application of the phosphodiesterase inhibitor IBMX blocks cAMP degradation and promotes the accumulation of basal cAMP. In epileptic control rats (August-Copenhagen Irish, ACI), but not in epileptic WAG/Rij, basal AC activity was sufficient to operate the molecular switch between the burst mode of sleep and epilepsy to the tonic relay mode (Budde et al. 2005). A number of studies indicated that environmental influences and antiepileptic drug application during an early postnatal period before P21 influences the occurrence of AE in later life (Schridde et al. 2006; Blumenfeld et al. 2008). This is exactly the postnatal age where altered HCN channel expression and cAMP sensitivity of I_h has been shown in WAG/Rij (Budde et al. 2005; Kuisle et al. 2006).

Based on these findings, we hypothesize that there are differences in AC gene and protein expression as well as in

AC enzymatic activity that contribute to the epileptic phenotype. Therefore, we compared data from the epileptic WAG/Rij strain to results derived from its corresponding control strain ACI for which no or only a few epileptic seizures are shown in the EEG (Inoue et al. 1990; Depaulis and van Luijckelaar 2006) and which are shown to be a reasonable control group (Budde et al. 2005; Broicher et al. 2007a, b, 2008; Kanyshkova et al. 2011). We have addressed AC signaling by combining quantitative real time (RT)-PCR, western blotting and determination of AC enzymatic activity. In order to account for the critical early postnatal period, we collected data at different developmental stages from the dorsal lateral geniculate nucleus (dLGN), a well-explored and prototypical specific thalamic nucleus. Results from the non-epileptic ACI control strain were compared to epileptic WAG/Rij rats to uncover AC-related mechanisms that might contribute to epilepsy-relevant, pathophysiological processes.

Experimental Procedures

All experimental procedures were carried out in accordance with the local authorities (review board institution: Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen; approval ID: 8.87-51.05.2010.117, 8.87-51.04.2010.A322).

Quantitative RT-PCR

Quantitative RT-PCR analysis was performed using dLGN from rats at different postnatal ages. Samples were collected using the laser capture technique (Zeiss/Palm MicroImaging). Total RNA was isolated using the RNeasy Micro Kit (Qiagen) and transcribed into cDNA using random primers (Fermentas) and the Sensiscript RT Kit (Qiagen). The hybridization primer/probe assays for RT-PCR detection were purchased from Applied Biosystems. As an internal standard, expression levels of 18 S rRNA were determined. The following intron spanning targets/constructs were used: AC-1, custom-assay for GenBank ID: NC_005113.2; AC-3, Rn00590729_m1; AC-8, Rn00691148_m1; and eukaryotic 18 S rRNA, 4319413E. Multiplex RT-PCR was performed using Quantitect Multiplex PCR Kit (Qiagen) and the ABI Prism 7000 Sequence Detection System (Applied Biosystems). The PCR program was set up for 15 min, 95 °C; 50 \times (1 min, 95 °C, 1 min, 60 °C). Results were analyzed with the ABI Prism 7000 SDS software. The primer efficiencies were nearly identical. C_t values for each sample were determined three times and included in the study with a standard error ≤ 0.16 . Quantification was done using the ΔC_t or $\Delta\Delta C_t$ method (Broicher et al. 2008). A minimum of $n=3$ out of three individual animals was generated per group. The

results were compared with Prism (GraphPad) using a two-way ANOVA.

Western Blotting

Extracts from dLGN of rats were prepared. A total amount of 2.5–5 μg of protein per lane were separated on 10 % SDS polyacrylamide gels and blotted onto nitrocellulose membranes. Unspecific binding sites were blocked with 5 % low-fat dry milk in PBS for 1 h at RT and then membranes were incubated overnight at 4 °C with either rabbit anti-AC-1 antibodies (1:500, Acris antibodies) or goat anti-AC-8 (1:150, Santa Cruz Biotechnology). As a loading control, β -actin (1:4,000; Abcam) was stained. Primary antibodies were detected with appropriate horseradish peroxidase-conjugated secondary antibodies (1:1,500–2,000; DAKO). Chemiluminescence was developed using the ECLplus kit (Amersham Biosciences). For analysis, Quantity One software (BioRad) was used. Statistical analysis was done with OriginPro8G (OriginLab) or Prism (GraphPad) using an unpaired Student *t* test for parametrically distributed data sets and Mann–Whitney test for non-parametrically distributed data sets.

Determination of cAMP Level in Tissue Samples

dLGN samples were prepared from freshly dissected coronal sections. Tissue was incubated in an oxygenated salt solution (in millimolars: NaCl, 125; KCl, 2.5; NaH_2PO_4 , 1.25; HEPES, 30; glucose, 10; CaCl_2 , 2; MgCl_2 , 1; and IBMX, 0.05) for 2 h. For AC activation, 50 μM of the water-soluble forskolin derivative NKH477 and for AC inhibitor, 200 μM SQ22536 was added to the incubation solution, respectively. Thereafter, sample preparation and cAMP level detection were done according to the protocol described in Kanyshkova et al. (2009). Statistical analysis was done with OriginPro8G (OriginLab) or Prism (GraphPad) using an unpaired Student *t* test for parametrically distributed data sets and Mann–Whitney test for non-parametrically distributed data sets.

Results

Quantification of AC Genes Transcription Levels

Since it has been suggested that Ca^{2+} -regulated ACs play an important role for the control of thalamic activity patterns (Bal and McCormick 1996; Lüthi and McCormick 1999; Kanyshkova et al. 2009), the expression profile of Ca^{2+} /CaM-activated ACs (AC-1, AC-3, and AC-8) were analyzed in a well-explored and prototypical specific thalamic nucleus (dLGN) of ACI and WAG/Rij rats by means of

quantitative RT-PCR. We analyzed AC expression at different developmental stages (P7–10, P21, P84) and calculated ΔC_t values (Fig. 1a; note that low/high ΔC_t values indicate high/low gene expression levels).

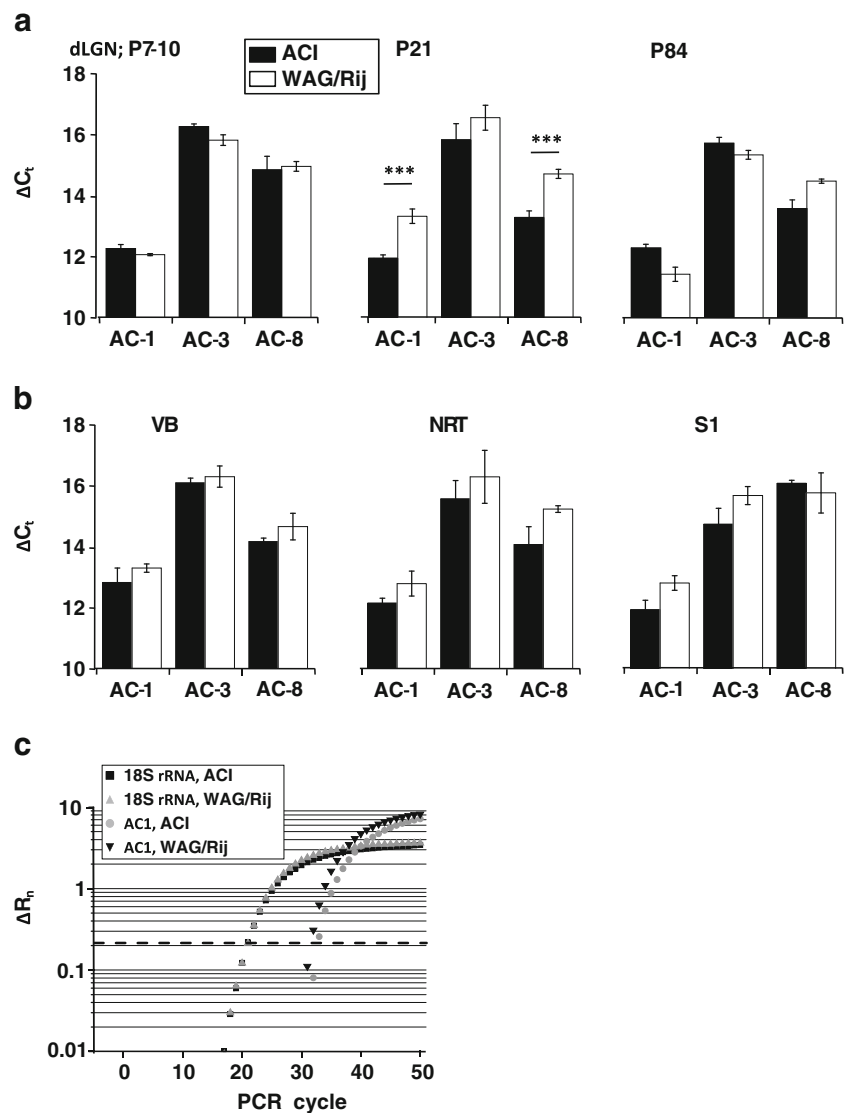
Early postnatal (P7–10) AC-1 gene expression showed the highest transcription level compared to AC-3 and AC-8. At this stage, there were no significant differences for the three subtypes between the two rat strains (Fig. 1a). At P21, a striking down-regulation of AC-1 and AC-8 was detected for epileptic WAG/Rij rats ($p < 0.001$). At adult stages (P84), these significant differences disappeared, and the expression levels of AC-1, AC-3, and AC-8 did not differ between the strains. This data set indicated a critical phase at P21 since two out of three Ca^{2+} /CaM-sensitive isoforms had a reduced expression level in WAG/Rij at this developmental stage. To further investigate this time point and to assess the involvement of ACs in the pathophysiology of AE, AC gene expression was also quantified in two other thalamic and one cortical region: ventrobasal thalamic complex (VB), nucleus reticularis thalami (NRT), and primary somatosensory cortex (S1). In aspect of AE, these regions are of great interest since previous studies indicated a major contribution to the initiation and propagation of spike-wave discharges typical in AE (Meeren et al. 2002; Pinault and O'Brien 2005; Steriade 2005). The data revealed no significant difference between the strains for the tested AC isoforms (Fig. 1b). For calculation of C_t values, the threshold (dashed line) was automatically set to the exponential phase of target amplification (Fig. 1c).

In general, the AC-1 isoform was found to be the most prominent one in all tested brain regions of both ACI and WAG/Rij rats. At pre-epileptic ages (P21), the mRNA expression levels of two Ca^{2+} -regulated AC isoforms were higher in the dLGN of non-epileptic compared to epileptic rats. Analysis of AE-associated brain regions in thalamus and cortex did not reveal striking differences between epileptic WAG/Rij and their corresponding controls. Therefore, the following experiments focused on dLGN, in order to assess differences in AC protein expression and enzymatic activity.

AC-1 and AC-8 Protein Expression in dLGN of ACI and WAG/Rij

As AC-1 and AC-8 have been suggested to represent the only AC isoforms stimulated by Ca^{2+} /CaM in vivo (Visel et al. 2006), we determined protein expression levels of the two isoforms by means of semi-quantitative western blotting. dLGN tissues at different developmental stages (P7–10, P21, and P84) were analyzed by using β -actin as house-keeping protein ($n=3/\text{group}$). For AC-1 of ACI rats, the analysis of the average intensity of protein bands revealed an AC-1/ β -actin ratio >1 , which was accompanied by a steady decrease of AC-1 expression until adulthood. AC-1

Fig. 1 Developmental expression of different AC genes in distinct brain tissue of non-epileptic controls (ACI) and epileptic WAG/Rij rats revealed by quantitative RT-PCR. **a, b** Expression profiles of the Ca^{2+} /CaM-stimulated isoforms AC-1, AC-3, and AC-8 in dLGN at P7–10, P21, and P84 (**a**) and in VB, NRT, and S1 at P21 (**b**) are shown. ΔC_t corresponds to the difference in threshold cycles for target and reference (18 S rRNA). Note that low ΔC_t values indicate high gene expression levels and vice versa. Significant differences between strains are marked. *dLGN* lateral geniculate nucleus, *VB* ventrobasal thalamic complex, *NRT* nucleus reticularis thalami, *S1* primary somatosensory cortex. Analysis was performed using two-way ANOVA; triple asterisks indicate $p < 0.001$. **c** Example of a quantitative RT-PCR experiment, analyzing the AC-1 mRNA expression in relation to 18 S rRNA. The number of PCR cycles is plotted vs. normalized and baseline-corrected reporter dye fluorescence. Dashed horizontal line marks the threshold used for quantification. Data were derived from a WAG/Rij rat



expression in WAG/Rij rats was comparable to that in ACI, displaying a maximum at P21 (Fig. 2a). The AC-8/ β -actin ratios of dLGN from both rat strains were low (≤ 1) at P7–10 and P21, and showed a clear nominal increase at adult stages (Fig. 2b).

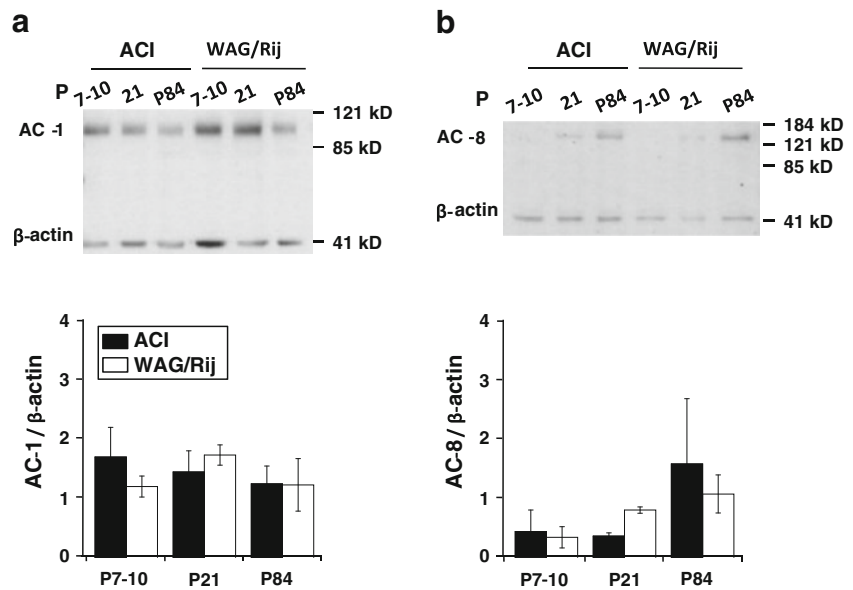
These findings support mRNA data that identified AC-1 as the prominent isoform in dLGN neurons in the two rat strains. However, no significant differences in protein expression were found between epileptic and non-epileptic animals.

Detection of cAMP Levels in Brain Tissue

Next, we assessed the basal, SQ22536-inhibited and NKH477-stimulated AC activities in dLGN at P7–10, P21, and P84 ($n=7-8$ /group). Basal cAMP concentrations were highest at P7–10 and strongly decreased in older animals, with no significant differences between rat strains. Blockade

of AC activity by SQ22536 (200 μM) and resulting decrease in cAMP levels had only a nominal effect in dLGN from ACI at P7–10. Furthermore, the forskolin derivative NKH477 (50 μM) induced a significant increase in cAMP levels at P21 and P84 in ACI ($p < 0.01$; Fig. 3a top) and at all developmental stages in WAG/Rij ($p < 0.05$; Fig. 3a bottom). The NKH477 effect revealed an intriguing developmental pattern with high enzymatic AC activity at P7–10 and P84 and a clear minimum at P21. The nominally stronger NKH477-stimulation in ACI did not reach statistical significance. This data points to a developmental down-regulation of rather low basal AC activity and an enormous enzymatic reserve that can be activated. At P21 the percentual increase of cAMP based on NKH477 application also revealed this large, up to sevenfold boost of cAMP production in dLGN from three weeks old animals (Fig. 3b). Although NKH477 strongly stimulated AC activity in tissue of both strains,

Fig. 2 AC expression in thalamic dLGN. **a, b** Western blot analysis of isoforms AC-1 and AC-8. Specific antibodies were used for AC-1 (**a**) and AC-8 (**b**) protein detection. Protein bands were detected at the calculated size of 108 and 139 kDa for AC-1 and AC-8, respectively



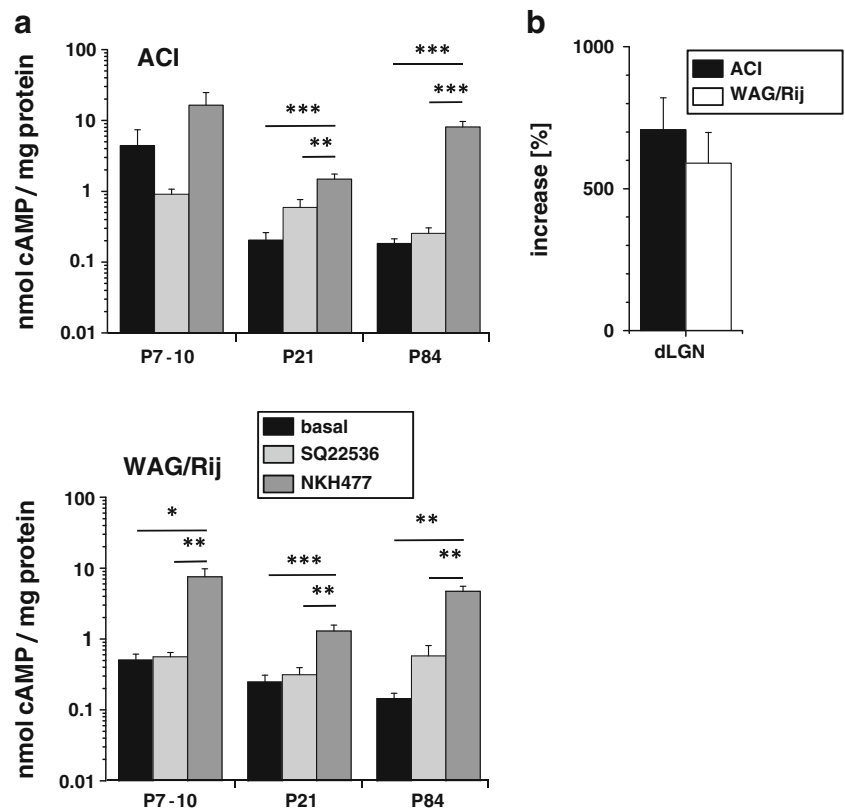
samples from non-epileptic rats showed only a nominally stronger cAMP production.

Discussion

The present study was undertaken to assess the expression and activity of different AC isoforms in the thalamus of

epileptic WAG/Rij and non-epileptic ACI rats. The results can be summarized as follows: (1) AC-1, AC-3, and AC-8 are expressed in thalamic dLGN, VB, and NRT as well as in cortical S1. (2) In all tested brain regions, the isoform AC-1 displayed the highest expression at mRNA level. (3) Basal AC activity was seemingly low, as was indicated by the insignificant effect of AC inhibition on cAMP levels. However, cAMP production was massively enhanced by

Fig. 3 AC enzymatic activity. **a** Basal, SQ22536-inhibited, and NKH477-stimulated AC activities in dLGN of ACI (*top*) and WAG/Rij rats (*bottom*). **b** Direct comparison of percentage increase in cAMP levels after NKH477 stimulation of AC activities in dLGN at P21. Single, double, and triple asterisks indicate $p < 0.5$, $p < 0.01$, and $p < 0.001$, respectively



NKH477 stimulation. (4) Strikingly, there were highly significant differences in AC-1 and AC-8 mRNA expression between the two rat strains in dLGN at P21. However, no differences were found for protein expression as well as enzymatic activity. Although differences in the complex subcellular cAMP signaling cannot be excluded, the data presented here indicate that differences in AC expression and activity per se may have only limited impact on the epileptic phenotype of WAG/Rij rats.

ACs in the Thalamus

Previous studies indicated that the strongest changes in AC mRNA expression pattern occur between P7 and P14 in rodent thalamus and thereafter rapidly approach adult levels (Matsuoka et al. 1997; Ihnatovych et al. 2002; Visel et al. 2006). It has therefore been suggested that thalamic ACs develop their final functionality in the early postnatal stage. One major isoform, namely AC-1, shows an expression peak at P10 followed by a gradual decrease to adult levels. AC-2 represents another major brain isoform that shows moderate changes in expression pattern throughout development but seems to have low expression in dLGN. The isoforms AC-5, AC-6, and AC-8 show moderate to weak expression at P7 and the adult stage, respectively. Furthermore, AC-4 does not seem to be expressed in neurons (Visel et al. 2006). Taken together, data from literature as well as findings presented herein point to a dominant function of AC-1 in the thalamus. It should be noted that immunoblotting revealed a hyperbolic increase in protein expression of several AC isoforms in thalamic tissue, indicating that measurements of mRNA levels and AC activity seem to dissociate (Ihnatovych et al. 2002). The reason for this remains as yet unresolved. Nevertheless, mRNA and protein expression data in this study also lacked high correlation.

The results of the present study demonstrate a complex AC expression pattern in dLGN and are generally in line with the findings described above: (1) AC-1 is the dominant isoform in both rat strains. While AC-1, AC-3, and AC-8 gene expression of young (P7–10) and adult rats (P84) revealed no differences between epileptic WAG/Rij and their non-epileptic controls, AC-1 and AC-8 showed a peculiar expression minimum at P21 in WAG/Rij. Co-expression of three genes (AC-1, AC-3, and AC-8) of the same group (Ca^{2+} /CaM-regulated ACs) in dLGN, suggests functional redundancy. This supports a scenario for cAMP signaling that depends on the combined action of multiple AC isoforms. (2) The results from cAMP assaying presented here correlate with previous findings by demonstrating highest levels at P7–10. Although mRNA data indicate reduced expression of AC-1 and AC-8 in epileptic WAG/Rij, there were no significant differences found for isoform-specific western blot analysis as well as for isoform-unspecific AC enzymatic activity.

Functional Aspects of AC Expression

The data described here point to a predominant role of AC-1 among Ca^{2+} /CaM-sensitive isoforms. All AC isoforms are activated by $G\alpha_s$ in vitro (Defer et al. 2000), with AC-1 being most easily stimulated (Harry et al. 1997). From in vivo studies, there is some evidence that AC-1 and AC-8 are insensitive to $G\alpha_s$ stimulation alone (Wayman et al. 1994). As such especially AC isoforms of the Ca^{2+} /CaM-stimulated subgroup were found to act as coincidence detectors in signaling cascades of associative learning and memory. Being a highly conserved mechanism, homologous AC isoforms, like the *rutabaga* AC in the invertebrate *Drosophila melanogaster* (Roman and Davis 2001) up to the mammalian AC-1 in rodents (Conti et al. 2007), can couple Ca^{2+} and neurotransmitter signals to generate optimal intracellular cAMP levels that convey the integrated signal inside the cell. However, there is massive AC stimulation during synergistic activation by $G\alpha_s$ and Ca^{2+} which is also true for AC-1 in thalamic relay neurons that are depolarized and at the same time have neurotransmitters bound to their membrane receptors. This situation is given when tonic firing is elicited in TC neurons during release of noradrenaline by the ascending reticular activating system. Moreover, typical $G\alpha_s$ -coupled receptors, like 5-HT_{7A}, activate AC-1 and AC-8 by increasing the intracellular Ca^{2+} concentration in hypothalamic neurons (Baker et al. 1998). A similar $G\alpha_s$ -dependent elevation of the intracellular Ca^{2+} concentration might occur in TC neurons. These types of co-incidence detection may be of relevance for the development of retinal projections (Nicol et al. 2006).

Conclusion

One aspect of the present study was to assess the possible involvement of ACs in the pathogenesis of AE. So far, only little is known about a possible malfunction of these enzymes in the development of epileptic activity. Nevertheless, as being part of the β -adrenergic/cyclic AMP/PKA pathway, ACs act as central players in intracellular signaling cascades in which they detect and integrate numerous signals. Former studies in TC neurons of epileptic WAG/Rij rats provided first hints that the endogenous cAMP synthesis is not sufficient to induce the switch between burst and tonic firing, whereas cAMP application did so (Budde et al. 2005). Data presented herein show a complex regulation of AC gene and protein expression throughout the development of the epileptic WAG/Rij rats as well as the non-epileptic ACI strain. However, at P21, significant differences in gene expression could be found in comparison of the two strains (see Fig. 1). Enhanced Ca^{2+} influx through T-type Ca^{2+} channels has been implicated in augmented transcription factor activity in mouse models of spike-wave

discharges (Ishige et al. 2001), and therefore it was proposed that these alterations in gene expression could affect AC gene transcription levels as well (Kuisle et al. 2006). However, data presented herein revealed only nominal lower AC activity in WAG/Rij rats. Taken together, our findings approve that AC functioning and thereby cAMP production does not contribute to the absence epileptic phenotype in WAG/Rij rats.

Acknowledgments Thanks are due to S. Balfanz, E. Naß, and K. Foraita for excellent technical assistance. This work was supported by DFG (BU 1019/8-1/9-2) and IZKF (Bud3/010/10).

References

- Baker LP, Nielsen MD, Impey S, Metcalf MA, Poser SW, Chan G, Obrietan K, Hamblin MW, Storm DR (1998) Stimulation of type 1 and type 8 Ca²⁺/calmodulin-sensitive adenylyl cyclases by the Gs-coupled 5-hydroxytryptamine subtype 5-HT_{7A} receptor. *J Biol Chem* 273:17469–17476
- Bal T, McCormick DA (1996) What stops synchronized thalamocortical oscillations? *Neuron* 17:297–308
- Biella G, Meis S, Pape H-C (2001) Modulation of a Ca²⁺-dependent K⁺-current by intracellular cAMP in rat thalamocortical relay neurons. *Thalamus Relat Syst* 1:157–167
- Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughinghouse C, Wang F, Phadke A, Mission J, Agarwal RK, Englot DJ, Motelow J, Nersesyan H, Waxman SG, Levin AR (2008) Early treatment suppresses the development of spike-wave epilepsy in a rat model. *Epilepsia* 49:400–409
- Broicher T, Kanyshkova T, Landgraf P, Rankovic V, Meuth P, Meuth SG, Pape H-C, Budde T (2007a) Specific expression of low-voltage-activated calcium channel isoforms and splice variants in thalamic local circuit interneurons. *Mol Cell Neurosci* 36:132–145
- Broicher T, Kanyshkova T, Meuth P, Pape H-C, Budde T (2008) Correlation of T-channel coding gene expression, IT, and the low threshold Ca²⁺ spike in the thalamus of a rat model of absence epilepsy. *Mol Cell Neurosci* 39:384–399
- Broicher T, Seidenbecher T, Meuth P, Munsch T, Meuth SG, Kanyshkova T, Pape H-C, Budde T (2007b) T-current related effects of antiepileptic drugs and a Ca²⁺ channel antagonist on thalamic relay and local circuit interneurons in a rat model of absence epilepsy. *Neuropharmacology* 53:431–446
- Budde T, Caputi L, Kanyshkova T, Staak R, Abrahamczik C, Munsch T, Pape H-C (2005) Impaired regulation of thalamic pacemaker channels through an imbalance of subunit expression in absence epilepsy. *J Neurosci Off J Soc Neurosci* 25:9871–9882
- Conti AC, Maas JW, Muglia LM, Dave BA, Vogt SK, Tran TT, Rayhel EJ, Muglia LJ (2007) Distinct regional and subcellular localization of adenylyl cyclases type 1 and 8 in mouse brain. *Neuroscience* 146:713–729
- Defer N, Best-Belpomme M, Hanoune J (2000) Tissue specificity and physiological relevance of various isoforms of adenylyl cyclase. *Am J Physiol Renal Physiol* 279:F400–F416
- Depaulis A, van Luijtelaar G (2006) Genetic Models of absence epilepsy in the rat. In: Pitkänen A, Schwartzkroin PA, Moshé SL (eds) *Models of seizures and epilepsy*. Elsevier Academic Press, San Diego, pp 223–232
- Frère SGA, Lüthi A (2004) Pacemaker channels in mouse thalamocortical neurones are regulated by distinct pathways of cAMP synthesis. *J Physiol* 554:111–125
- Harry A, Chen Y, Magnusson R, Iyengar R, Weng G (1997) Differential regulation of adenylyl cyclases by Galphas. *J Biol Chem* 272:19017–19021
- Ihnatovych I, Novotny J, Haugvicova R, Bourova L, Mares P, Svoboda P (2002) Ontogenetic development of the G protein-mediated adenylyl cyclase signalling in rat brain. *Brain Res Dev Brain Res* 133:69–75
- Inoue M, Peeters BW, van Luijtelaar EL, Vossen JM, Coenen AM (1990) Spontaneous occurrence of spike-wave discharges in five inbred strains of rats. *Physiol Behav* 48:199–201
- Ishige K, Endo H, Saito H, Ito Y (2001) Repeated administration of CGP 46381, a gamma-aminobutyric acidB antagonist, and ethosuximide suppresses seizure-associated cyclic adenosine 3'5' monophosphate response element- and activator protein-1 DNA-binding activities in lethargic (lh/lh) mice. *Neurosci Lett* 297:207–210
- Kanyshkova T, Meuth P, Bista P, Liu Z, Ehling P, Caputi L, Döngi M, Chetkovich DM, Pape H-C, Budde T (2011) Differential regulation of HCN channel isoform expression in thalamic neurons of epileptic and non-epileptic rat strains. *Neurobiol Dis* 45:450–461
- Kanyshkova T, Pawlowski M, Meuth P, Dubé C, Bender RA, Brewster AL, Baumann A, Baram TZ, Pape H-C, Budde T (2009) Postnatal expression pattern of HCN channel isoforms in thalamic neurons: relationship to maturation of thalamocortical oscillations. *J Neurosci Off J Soc Neurosci* 29:8847–8857
- Kuisle M, Wanaverbecq N, Brewster AL, Frère SGA, Pinault D, Baram TZ, Lüthi A (2006) Functional stabilization of weakened thalamic pacemaker channel regulation in rat absence epilepsy. *J Physiol* 575:83–100
- Lüthi A, McCormick DA (1999) Modulation of a pacemaker current through Ca²⁺-induced stimulation of cAMP production. *Nat Neurosci* 2:634–641
- Matsuoka I, Suzuki Y, Defer N, Nakanishi H, Hanoune J (1997) Differential expression of type I, II, and V adenylyl cyclase gene in the postnatal developing rat brain. *J Neurochem* 68:498–506
- McCormick DA, Huguenard JR (1992) A model of the electrophysiological properties of thalamocortical relay neurons. *J Neurophysiol* 68:1384–1400
- Meeren HKM, Pijn JPM, Van Luijtelaar ELJM, Coenen AML, Lopes da Silva FH (2002) Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci Off J Soc Neurosci* 22:1480–1495
- Meuth S, Pape H-C, Budde T (2002) Modulation of Ca²⁺ currents in rat thalamocortical relay neurons by activity and phosphorylation. *Eur J Neurosci* 15:1603–1614
- Meuth SG, Kanyshkova T, Meuth P, Landgraf P, Munsch T, Ludwig A, Hofmann F, Pape H-C, Budde T (2006) Membrane resting potential of thalamocortical relay neurons is shaped by the interaction among TASK3 and HCN2 channels. *J Neurophysiol* 96:1517–1529
- Nicol X, Bennis M, Ishikawa Y, Chan GC-K, Repérant J, Storm DR, Gaspar P (2006) Role of the calcium modulated cyclases in the development of the retinal projections. *Eur J Neurosci* 24:3401–3414
- Pinault D, O'Brien TJ (2005) Cellular and network mechanisms of genetically-determined absence seizures. *Thalamus Relat Syst* 3:181–203
- Roman G, Davis RL (2001) Molecular biology and anatomy of *Drosophila* olfactory associative learning. *BioEssays News Rev Mol Cell Dev Biol* 23:571–581
- Schridde U, Strauss U, Brauer AU, van Luijtelaar G (2006) Environmental manipulations early in development alter seizure activity, Ih and HCN1 protein expression later in life. *Eur J Neurosci* 23:3346–3358

- Sherman SM (2007) The thalamus is more than just a relay. *Curr Opin Neurobiol* 17:417–422
- Steriade M (1997) Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. *Cerebral cortex* (New York, NY: 1991) 7:583–604
- Steriade M (2005) Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends Neurosci* 28:317–324
- Visel A, Alvarez-Bolado G, Thaller C, Eichele G (2006) Comprehensive analysis of the expression patterns of the adenylyl cyclase gene family in the developing and adult mouse brain. *J Comp Neurol* 496:684–697
- Wayman GA, Impey S, Wu Z, Kindsvogel W, Prichard L, Storm DR (1994) Synergistic activation of the type I adenylyl cyclase by Ca²⁺ and Gs-coupled receptors in vivo. *J Biol Chem* 269:25400–25405