

Astrogliosis in epilepsy leads to overexpression of adenosine kinase, resulting in seizure aggravation

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Adenosine kinase (ADK) is considered to be the key regulator of the brain's endogenous anticonvulsant, adenosine. In adult brain, ADK is primarily expressed in a subpopulation of astrocytes and striking upregulation of ADK in these cells has been associated with astrogliosis after kainic acid-induced status epilepticus (KASE) in the kainic acid mouse model of temporal lobe epilepsy. To investigate the causal relationship between KASE-induced astrogliosis, upregulation of ADK and seizure activity, we have developed a novel mouse model [the *Adktm1*^{-/-}-Tg(*UbiAdk*) mouse] lacking the endogenous astrocytic enzyme due to a targeted disruption of the endogenous gene, but containing an *Adk* transgene under the control of a human ubiquitin promoter. Mutant *Adktm1*^{-/-}-Tg(*UbiAdk*) mice were characterized by increased brain ADK activity and constitutive overexpression of transgenic ADK throughout the brain, with particularly high levels in hippocampal pyramidal neurons. This ADK overexpression was associated with increased baseline levels of locomotion. Most importantly, two-thirds of the mutant mice analysed exhibited spontaneous seizure activity in the hippocampus and cortex. This was the direct consequence of transgene expression, since this seizure activity could be prevented by systemic application of the ADK inhibitor 5-iodotubercidin. Intrahippocampal injection of kainate in the mutant mice resulted in astrogliosis to the same extent as that observed in wild-type mice despite the absence of endogenous astrocytic ADK. Therefore, KASE-induced upregulation of endogenous ADK in wild-type mice is a consequence of astrogliosis. However, seizures in kainic acid-injected mutants displayed increased intra-ictal spike frequency compared with wild-type mice, indicating that, once epilepsy is established, increased levels of ADK aggravate seizure severity. We therefore conclude that therapeutic strategies that augment the adenosine system after astrogliosis-induced upregulation of ADK constitute a neurochemical rationale for the prevention of seizures in epilepsy.

Keywords: adenosine; adenosine kinase; epilepsy; astrogliosis; transgenic mice

Abbreviations: AIR = adenosine A₁ receptor; ADK = adenosine kinase; DPCPX = 8-cyclopentyl-1,3-dipropyl-xanthine; EPSC = excitatory postsynaptic current; GFAP = glial fibrillary acidic protein; ITU = 5-iodotubercidin; KA = kainic acid; KASE = kainic acid-induced status epilepticus; LCCGI = (2*S*,1'*S*,2'*S*)-2-carboxycyclopropyl glycine; NBQX = 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulphonamide; Tg = transgenic

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Introduction

The purine ribonucleoside adenosine is an important modulator of central nervous system functions (Dunwiddie and Masino, 2001), exerting primarily inhibitory effects on neuronal activity. Adenosine receptor antagonists, such as caffeine, display stimulant properties (Fredholm *et al.*, 1999), while

activation of the adenosine A₁ receptor (A₁R) has potent inhibitory effects on neuronal activity (Fredholm, 1997). In humans, microdialysis studies demonstrated a release of adenosine during seizure activity and adenosine was proposed to be a natural mediator of seizure arrest and postictal

refractoriness (During and Spencer, 1992). Systemic administration of selective A1R agonists or intracerebral grafting of encapsulated adenosine-releasing cells suppresses seizure activity in various rodent models of epilepsy (Dunwiddie, 1999; Dunwiddie and Masino, 2001; Boison, 2005). Of particular relevance is the recent finding that activation of A1Rs leads to the suppression of seizures in the mouse kainate model of pharmacoresistant temporal lobe epilepsy, thus pointing to adenosine as a potential therapeutic advance compared with conventional antiepileptic drugs (Gouder et al., 2003). Due to the therapeutic potential of adenosine in the treatment of pharmacoresistant epilepsy, the study of mechanisms underlying adenosinergic neuromodulation in epilepsy is an important issue.

On the basis of its low K_M for adenosine, adenosine kinase (ADK, EC 2.7.1.20) is considered to be the key enzyme for the regulation of brain adenosine levels (Mathews et al., 1998). This concept is supported by a number of studies: (i) pharmacological inhibition of ADK increases A1R-mediated pre-synaptic inhibition in hippocampal slices (Pak et al., 1994); (ii) inhibition of adenosine deaminase has little or no effect on the extracellular adenosine concentration (Pak et al., 1994; Zhu and Krnjevic, 1994; Huber et al., 2001); and (iii) in the brain there is a high flux rate in the futile cycle of adenosine to AMP involving ADK and 5'-nucleotidase (Bontemps et al., 1983).

Normally, brain activity is under the influence of tonic adenosinergic inhibition. This results from low basal ADK levels produced by a subpopulation of astrocytes that are evenly distributed throughout the whole brain (Gouder et al., 2004). However, in the mouse kainate model of temporal lobe epilepsy, upregulation of endogenous astrocytic ADK is observed in parallel with ongoing astrogliosis and seizure activity after kainic acid (KA)-induced status epilepticus (KASE) (Gouder et al., 2004). To analyse the specific role of ADK in astrogliosis and seizure activity, we have created a novel mouse mutant in which the endogenous expression of ADK in astrocytes is abolished and replaced by constitutive transgene-driven overexpression of ADK.

Methods

Generation of adenosine kinase transgenic mice

Adktm1^{-/-} mice were created by homologous recombination of the *Adk* gene in embryonic stem cells as described (Boison et al., 2002). For the generation of *Adk* transgenic animals, an 1865 bp full-length *Adk* cDNA (Gouder et al., 2004), homologous to the short isoform (38.7 kDa) of *Adk* described in humans (McNally et al., 1997), was cloned into a transgene expression vector containing a human ubiquitin promoter and the splice and poly(A) sequences of SV40 (Schorpp et al., 1996). In a last cloning step, the *Adk* expression cassette was flanked with loxP sites. The linearized vector was purified and injected into the pronucleus of fertilized oocytes derived from *Adktm1^{+/-}* matings. Three independent *UbiAdk* transgenic lines were established and maintained on a mixed B6,129P3 background.

Animals

The introduction of the *UbiAdk* transgene into the *Adktm1^{-/-}* background rescued the lethal phenotype related to ADK deficiency (Boison et al., 2002). *Adktm1^{-/-}*-transgenic (Tg)(*UbiAdk*) mice from two of the three lines (Tg888 and Tg890) were fertile and did not show overt abnormality. Male *Adktm1^{-/-}*-Tg(*UbiAdk*) and wild-type mice, aged 8–9 weeks, were used for all *in vivo* analyses. They were reared in individual cages with food and water *ad libitum* under standard 12-h light/12-h dark cycle conditions (light on at 07:00 h). All animal procedures were conducted in accordance with the regulations of the local animal welfare authority. All efforts were made to minimize animal suffering and to reduce the number of animals used for experimentation.

Genotyping

The endogenous *Adk* locus was analysed in individual PCR reactions containing a mix of the three primers o107, o108 and o109 (Fedele et al., 2004). PCR primers used for the detection of the *Adk* transgene were o141, 5'-GGA AGC TGC CAC TTT TGC TAG AGA GC-3', and o142, 5'-GGA ACG CTC AGG ATG TGG TCA CTG C-3'. The combination of primers o107 with o108 gave rise to 640-bp products (indicative of a wild-type *Adk* allele), whereas the combination of primers o107 with o109 gave rise to 840-bp products (indicative of the *Adk* knockout allele). The combination of primers o141 and o142 gave rise to 420-bp products indicative of the transgene. The zygosity of the transgene was determined in a TaqMan™ real-time PCR analysis as follows. Individual TaqMan PCR reactions were performed in duplicate with the ABI PRISM™ 7700 Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland) as described previously (Zumsteg and Boison, 2002). For the quantification of the transgene, the following transgene specific primers were used: tg-fwd (5'-ACT TGT GCT GCG TGC ATC A-3'), tg-rev (5'-TAA CAA TTG GCG GCA GCA A-3'), and a 5'-FAM- and 3'-TAMRA-labelled fluorogenic TaqMan probe (5'-FAM-CAA CGA GGG ACC TGT TGC CAC CA-3'-TAMRA). Amplification of the *Adk* knockout allele was used as an internal reference [two allele copies in *Adktm1^{-/-}*-Tg(*UbiAdk*) mice]. The knockout-specific primers and probes were specific for the EGFP-containing gene insertion cassette: Adk-ko-fwd (5'-GCC GTC TTT TGG CAA TGT G-3'), Adk-ko-rev (5'-CCC CTA GGA ATG CTC GTC AA-3'), and a 5'-FAM- and 3'-TAMRA-labelled fluorogenic TaqMan probe (5'-FAM-CCG GGA AAC CTG GCC CTG TCT T-3'-TAMRA). After completion of the PCR reactions, a threshold of the fluorescence intensity was set within the linear phase of the amplifications. The cycle number (CT) in which this threshold was crossed by the active reporter fluorescence was determined. The zygosity was calculated by comparison of the CT values of the respective *tgUbiAdk*-allele with the CT values from the reference gene. All *Adktm1^{-/-}*-Tg(*UbiAdk*) mice used in this study were homozygous for the transgenic locus.

Western blot analysis

Aqueous extracts derived from whole brain, liver, lung, kidney and heart from adult *Adktm1^{-/-}*-Tg(*UbiAdk*) ($n = 3$) and wild-type mice ($n = 3$) were prepared as described (7). Supernatants, each containing 15 µg protein, were separated on a sodium dodecyl sulphate/10% polyacrylamide gel and blotted onto a nitrocellulose membrane. The blots were probed with a polyclonal rabbit antiserum against ADK and processed as described (Boison et al., 2002; Gouder et al.,

2004). Note that, in wild-type brain, only the short 38.7 kDa isoform of ADK is expressed, whereas in the other organs both isoforms are present. Because we used the short isoform of ADK for the transgenic rescue of ADK deficiency, the overexpression of ADK in *Adktm1*^{-/-}-Tg(*UbiAdk*) mice involves only this isoform.

In vitro electrophysiology

Acute coronal slices (300 µm) containing the hippocampus were obtained from P13–P20 *Adktm1*^{-/-}-Tg(*UbiAdk*) and wild-type mice. They were incubated for 30 min in oxygenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid solution at 34°C containing (mM) 124 NaCl, 2.5 KCl, 1.3 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, 2.5 CaCl₂ and 11 glucose, and maintained at room temperature for at least 1 h before they were transferred to a superfusing chamber. Whole-cell voltage-clamp recordings of CA3 pyramidal neurons were performed at room temperature in response to mossy fibre stimulation delivered via a bipolar tungsten stimulating electrode placed in the stratum lucidum (0.1 ms, 1–10 V, at 20-s interstimulus intervals). Voltage-clamp recordings were obtained with 3–5 MΩ patch pipettes containing (mM) 130 K⁺ gluconate, 10 HEPES, 1 EGTA, 5 NaCl, 5 ATP-Mg and 0.5 GTP-Na. Excitatory postsynaptic currents (EPSCs) were recorded at a holding potential of -70 mV in the presence of picrotoxin (100 µM) and a subsaturating concentration of NBQX (1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide) (0.25 µM) to prevent picrotoxin-induced seizure activity in slices from *Adktm1*^{-/-}-Tg(*UbiAdk*) mice. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) or 5'-iodotubercidin (ITU) was added at final concentrations of 200 nM or 5 µM, respectively. At the end of all experiments, (2S,1'S,2'S)-2-(carboxycyclopropyl) glycine (LCCG1) (10 µM) was added to confirm that EPSCs originated in mossy fibres (Moore *et al.*, 2003). Experiments were used only if the LCCG1-mediated inhibition was at least 70% of EPSC peak amplitude. Signals were recorded with an Axopatch 200B (Axon Instruments, Foster City, CA, USA) amplifier and digitized at 5 kHz. Data were processed using IgorPro software (Wavemetrics, Lake Oswego, OR, USA). Data were discarded if access resistance varied by more than 20% during the course of the experiment. Picrotoxin, NBQX, DPCPX and LCCG1 came from Tocris Cookson (Bristol, UK) and were prepared daily from concentrated stock solutions.

Circadian locomotor activity

Mutant and wild-type mice were transferred to the testing room and allowed to adapt to a reversed 12-h light/dark cycle (light on at 20:00 h) for at least 10 days before the experiment. Spontaneous locomotor activity was recorded continuously in automated individual circular arenas (Imetronic, Pessac, France) over a period of 4 days. Data were collected during the last 3 days for statistical analysis. After completion of the experiment, mice were killed by decapitation for ADK activity measurement.

Electroencephalographic recordings

Adktm1^{-/-}-Tg(*UbiAdk*) (*n* = 24) and wild-type (*n* = 9) mice were tested for spontaneous seizure activity. The procedure of electrode implantation was as previously described (Gouder *et al.*, 2003). In addition to the standard bipolar electrode in the dorsal hippocampus, 14 out of the 24 mice were equipped with two monopolar electrodes placed at the cortical surface for simultaneous hippocampal and cortical EEG recordings. The day after surgery, mice were familiarized with the recording chamber and EEG activities were

recorded as described previously (Gouder *et al.*, 2003). A further series of 6 *Adktm1*^{-/-}-Tg(*UbiAdk*) mice and six wild-type mice were stereotactically injected with a solution of 1 nmol of KA into the right dorsal hippocampus as described (Gouder *et al.*, 2004). All KA-injected mice were implanted with a bipolar electrode into the right dorsal hippocampus and a monopolar reference electrode over the cerebellum. They were subjected to EEG recordings 4–6 weeks after KA injection.

Histological analysis

Adult *Adktm1*^{-/-}-Tg(*UbiAdk*) and wild-type mice were perfused transcardially with 4% paraformaldehyde and 15% saturated picric acid solution in phosphate buffer (0.15 M, pH 7.4). The brains were then postfixed in the same fixative at 4°C for 6 h and cryoprotected in 10% dimethylsulphoxide in phosphate-buffered saline (v/v) before being cut into 40 µm sagittal or coronal sections using a sliding microtome. For the immunohistochemical detection of ADK, brain sections were incubated overnight at 4°C with primary anti-ADK antiserum diluted 1 : 5000 in Tris-Triton, pH 7.4, containing 2% normal goat serum and 0.2% Triton X-100. The sections were then washed 3 × 10 min in Tris-buffered saline (TBS) at pH 7.4, incubated for 30 min with biotinylated goat anti-rabbit antibody diluted 1 : 300 in Tris-Triton, pH 7.4, containing 2% normal goat serum, washed again three times in TBS then incubated for 20–30 min with an avidin : biotin enzyme complex (Vectastain Elite Kit; Vector Laboratories, Burlingame, CA, USA). After washing again three times with TBS, the tissue antigen was localized by incubation with diaminobenzidine hydrochloride (Sigma, St Louis, MO, USA), which acts as a chromogen. The sections were then mounted on gelatin-coated slides, air-dried and coverslipped. The immunoperoxidase-stained sections were analysed with a Zeiss AxioCam microscope (Zeiss, Jena, Germany) equipped with bright-field microscopy, using a high-resolution digital camera. KA-induced astrogliosis was evaluated by immunofluorescence staining with a monoclonal mouse antibody against the astrocytic marker glial fibrillary acidic protein (GFAP) (MAB360; Chemicon International, Temecula, CA, USA) on coronal brain sections from wild type and *Adktm1*^{-/-}-Tg(*UbiAdk*) mice, 6 weeks after KA injection. GFAP immunofluorescence staining was also performed on coronal sections from untreated control animals of both genotypes for comparison.

Adenosine kinase enzyme activity

Brain homogenates were purified and ADK activity was quantified using an enzyme-linked bioluminescent assay (Gouder *et al.*, 2004) according to the following principle: an excess of adenosine, added to the sample, is phosphorylated by ADK to AMP, which is then phosphorylated further to ATP by an excess of recombinant GTP : AMP phosphotransferase (own production) and pyruvate kinase. The resulting ATP is then quantified with a commercial luciferase assay kit (ATP Bioluminescent Assay Kit; Sigma). ADK activity values, expressed as the relative number of light units per time, were normalized to endogenous lactate dehydrogenase (LDH) activity, which was taken as an internal standard. LDH activity was determined with a commercial assay kit according to the manufacturer's protocol (Roche, Rotkreuz, Switzerland). The assay was validated by plotting a standard curve of different ratios of samples from wild-type mice mixed with those from *Adktm1*^{-/-} mice.

Results

Insertion of an *Adk* transgene provides genetic rescue of lethal ADK-deficiency

To study the causal relationship between overexpression of ADK, astrogliosis and seizure activity, we changed the expression of ADK from selected astrocytes to a widespread expression pattern encompassing both neurons and astrocytes. This was accomplished by introducing an ubiquitously expressed *Adk* transgene into an ADK-deficient host mouse line (*Adktm1*^{-/-}) (Boison et al., 2002). We constructed a transgene (*tgUbiAdk*) of an *Adk* cDNA (Boison et al., 1999), corresponding to the short (38.7 kDa) brain-specific isoform of the enzyme (McNally et al., 1997; Gouder et al., 2004), under the control of the human ubiquitin promoter (Fig. 1A) (Schorpp et al., 1996). The transgene was injected into pronuclei of fertilized oocytes derived from *Adktm1*^{-/-} matings. Twenty-nine mice were obtained, of which three founders carried the transgene on an *Adktm1*^{+/-} background (*Adktm1*^{+/-}-*Tg(UbiAdk)*, Fig. 1B). These mice were then bred with *Adktm1*^{+/-} mice. Two of the three transgenic lines (Tg888 and Tg890) gave rise to all six possible genotypes (Fig. 1B), including *Adktm1*^{-/-}-*Tg(UbiAdk)*. *Adktm1*^{-/-}-*Tg(UbiAdk)* mice (m#3, Fig. 1C) were identified in an *Adk*-specific PCR analysis by a characteristic 840 bp band from the *Adk* knockout allele (m#2 in Fig. 1C as control), a lack of the 640 bp band indicative of the wild-type allele (m#1 in Fig. 1C as control), and the presence of a 420 bp band (m#3 in Fig. 1C) from the *UbiAdk* transgene. The genotypes were also verified in a transgene- and knockout-specific TaqMan real-time PCR analysis (data not shown).

Overexpression of brain ADK in *Adktm1*^{-/-}-*Tg(UbiAdk)* mice

The expression of the *tgUbiAdk* transgene was examined by Western blot analysis in various organs from adult *Adktm1*^{-/-}-*Tg(UbiAdk)* (line Tg888) and wild-type mice. In wild-type mice, ADK was highly expressed in liver and intermediate levels were observed in brain, lung and kidney, and the weakest expression was found in the heart (Fig. 2A). In *Adktm1*^{-/-}-*Tg(UbiAdk)* mice, the highest expression of ADK was detected in brain extracts, whereas the liver ADK content was poorly reconstituted (Fig. 2A). ADK levels in heart, kidney and lung of the mutants were similar to those in wild-type mice. Remarkably, the rather low levels of transgenic ADK expression in liver appeared to be sufficient for phenotypic correction of the *Adk*^{-/-} fatty liver (data not shown). We quantified ADK enzyme activity using an enzyme-coupled bioluminescent assay in brain extracts from mutant and wild-type mice and found that *Adktm1*^{-/-}-*Tg(UbiAdk)* mice displayed a 2.2-fold increase in ADK activity compared with wild-type mice ($P < 0.001$, median test) (Fig. 2B). In addition, we measured brain ADK activity in three *Adktm1*^{-/-}-*Tg(UbiAdk)* mice from line Tg890 and observed a slight elevation (6%) compared with wild-type

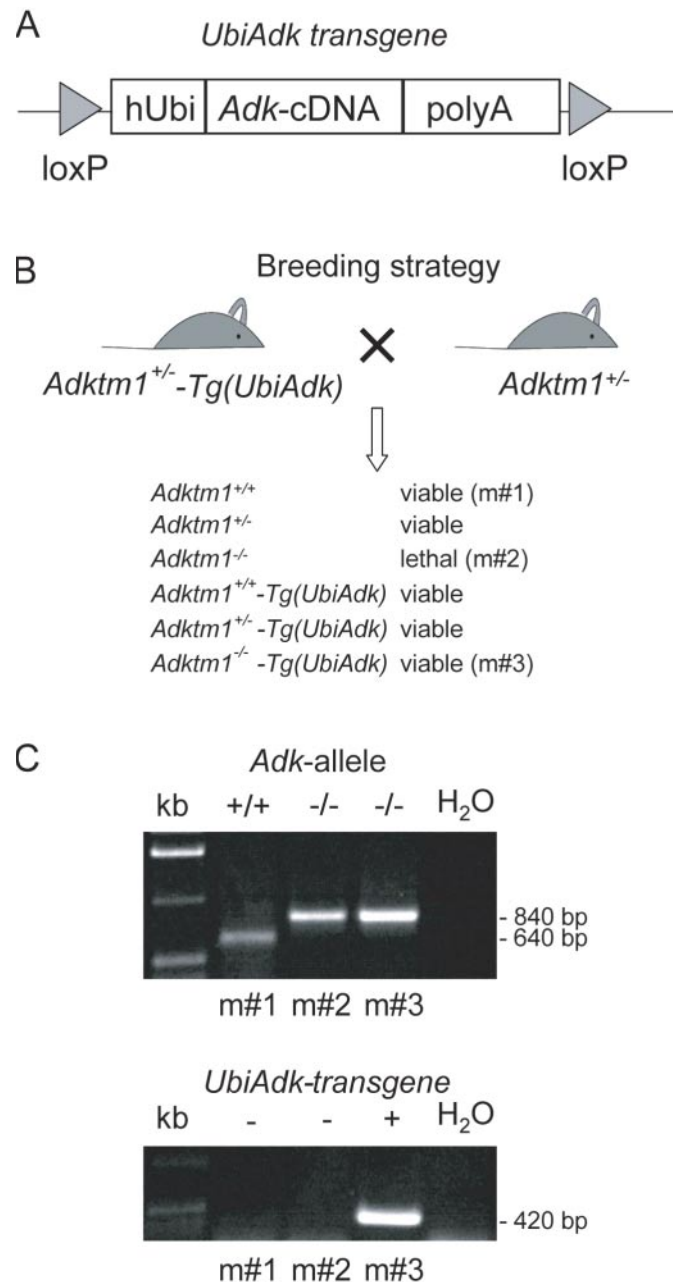


Fig. 1 Generation of *Adktm1*^{-/-}-*Tg(UbiAdk)* mice. **(A)** The short isoform of mouse *Adk*-cDNA was inserted between a human ubiquitin promoter (hUbi) and an SV40 poly(A) sequence (polyA). The *UbiAdk* transgene (open boxes) was flanked by loxP sites (grey triangles). **(B)** Transgenic founders, *Adktm1*^{+/-}-*Tg(UbiAdk)* mice, which were heterozygous for both the transgene and the *Adk* knockout, were backcrossed with *Adktm1*^{+/-} mice. From these backcrosses, offspring with six different genotypes were derived, among them *Adktm1*^{-/-}-*Tg(UbiAdk)* mice (m#3), which were viable, like wild-type mice (*Adk*^{+/+}) (m#1), and in contrast to homozygous *Adk* knockout mice, which die shortly after birth (*Adktm1*^{-/-}, m#2). **(C)** Representative PCR analysis of three selected animals (m#1–3). (Top) The *Adk* wild-type allele (+/+) in m#1 is observed as a 640 bp band, while the knockout allele (-/-) in m#2 and m#3 is observed as an 840 bp band. (Bottom) The *UbiAdk* transgene-specific PCR gave rise to a characteristic amplification product of 420 bp only in m#3; the transgene was not detectable in m#1 and m#2.

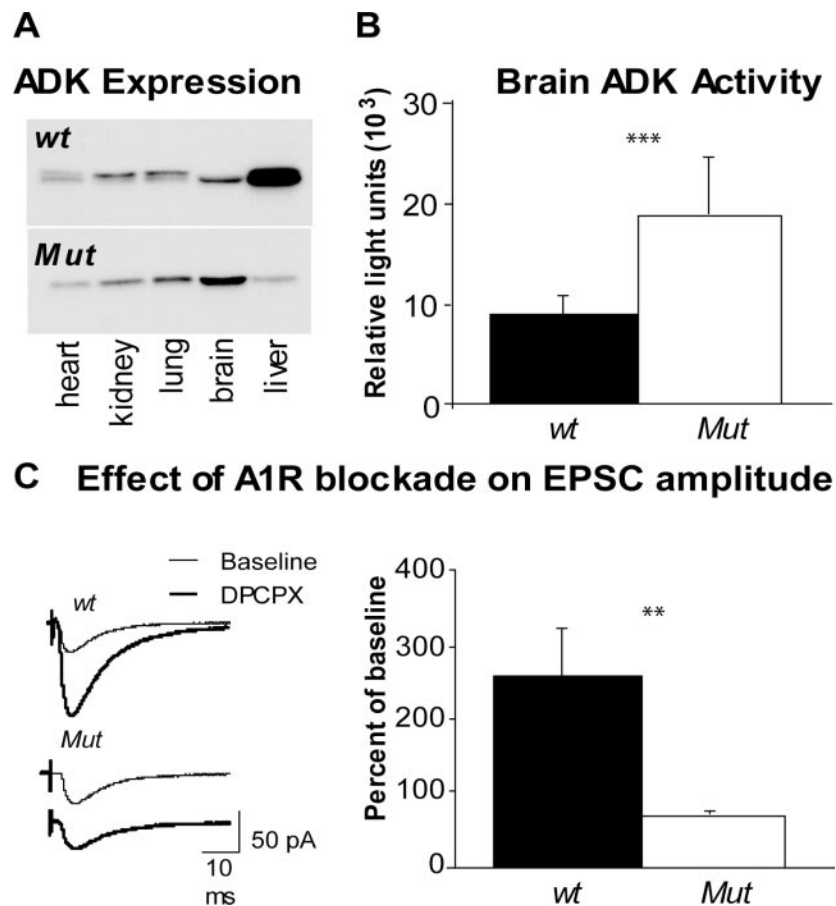


Fig. 2 Increased brain ADK expression and activity in *Adktm1*^{-/-}-Tg(*UbiAdk*) mice. **(A)** Western blot analysis of aqueous protein extracts derived from various organs from adult wild-type (wt) and *Adktm1*^{-/-}-Tg(*UbiAdk*) mice (Mut). Extracts corresponding to 15 μ g of total protein each were probed with polyclonal rabbit antiserum raised against recombinant mouse ADK. Note the increase in ADK expression in the brain of *Adktm1*^{-/-}-Tg(*UbiAdk*) mice compared with wild type. **(B)** Brain ADK enzyme activity, as measured using an enzyme-coupled bioluminescent assay, was increased in *Adktm1*^{-/-}-Tg(*UbiAdk*) mice ($n = 6$) in comparison to wild-type mice ($n = 7$; *** $P < 0.001$, median test). Results are presented as relative activities and expressed as mean \pm SE. **(C)** EPSC traces and EPSC response amplitudes from whole-cell recordings of CA3 hippocampal pyramidal neurons in acute slices from wild-type ($n = 4$) and *Adktm1*^{-/-}-Tg(*UbiAdk*) mice ($n = 6$), before and during application of DPCPX (200 nM). The EPSC response amplitudes were normalized to each individual baseline. Values are expressed as the mean percentages EPSC amplitude of baseline \pm SE (** $P < 0.01$, Mann–Whitney test).

mice. All further studies described here were therefore pursued in *Adktm1*^{-/-}-Tg(*UbiAdk*) mice of line Tg888.

Novel neuronal expression of ADK in *Adktm1*^{-/-}-Tg(*UbiAdk*) mouse brain

The expression pattern of ADK was analysed by immunoperoxidase staining performed on coronal and sagittal sections from adult *Adktm1*^{-/-}-Tg(*UbiAdk*) and wild-type mice ($n = 3$ per group). In wild-type coronal sections, strong ADK immunoreactivity was detected in evenly distributed astrocytes (Fig. 3A, C, E) whereas neurons, such as pyramidal cells of the CA3 region (Fig. 3C) and granular neurons of the dentate gyrus (Fig. 3E), lacked specific ADK immunoreactivity. In contrast, in *Adktm1*^{-/-}-Tg(*UbiAdk*) mice the deletion of the endogenous *Adk* gene was reflected by a striking loss of endogenous astrocytic ADK immunoreactivity, which was replaced by rather diffuse and ubiquitous expression of

the *UbiAdk* transgene in both neurons and astrocytes (Fig. 3B). Since all brain sections were stained under identical conditions, the darker staining in the mutant sections indicates diffuse overexpression of ADK compared with wild type (Fig. 3A, B). While in wild-type animals ADK immunoreactivity was nearly evenly distributed among different brain regions, in mutant animals ADK immunoreactivity appeared to be slightly higher in cortical brain regions compared with more basal brain regions. In the hippocampal formation of *Adktm1*^{-/-}-Tg(*UbiAdk*) brain sections, transgene-induced neuronal expression of ADK became particularly evident in CA1 and CA3 pyramidal cells (Fig. 3B, D) as well as in neurons of the hilus (Fig. 3F). A similar shift from astrocytic to ubiquitous ADK immunoreactivity was observed in the cerebellum from *Adktm1*^{-/-}-Tg(*UbiAdk*) sagittal sections (data not shown). Thus, the *Adktm1*^{-/-}-Tg(*UbiAdk*) mice are characterized by novel expression of ADK in neurons.

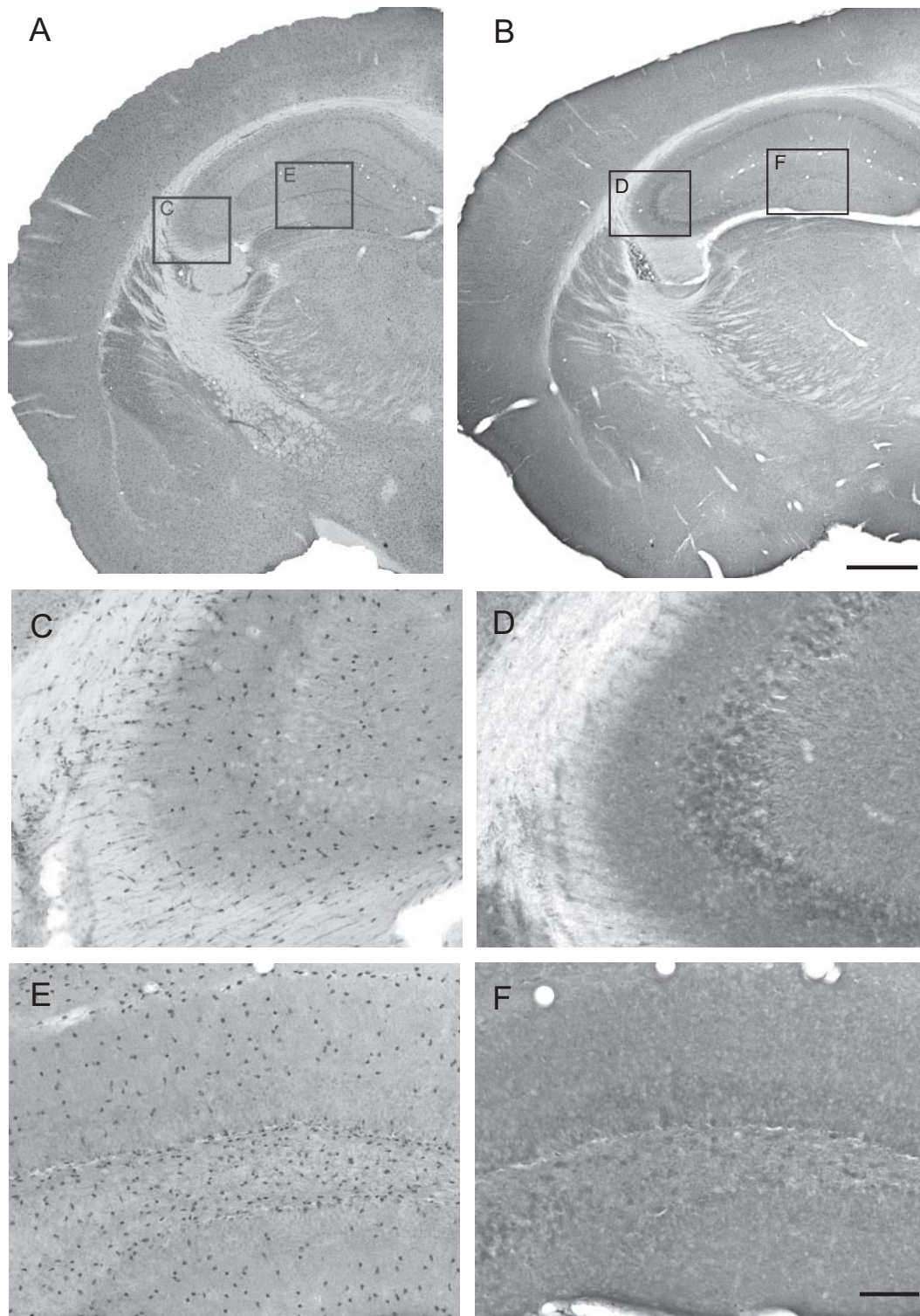


Fig. 3 Photomicrographs of ADK immunoreactivity in coronal brain sections of adult mice, processed for immunoperoxidase staining under identical conditions. **(A)** Coronal section of a wild-type brain hemisphere showing homogeneous distribution of individual cells expressing ADK. **(B)** Transverse section of an *Adktm1*^{-/-}-Tg(*UbiAdk*) brain hemisphere showing ubiquitous expression of transgenic ADK coupled to a loss of punctate staining of endogenous ADK in individual cells. **(C)** CA3 region of a wild-type hippocampus (see inset in **A**) at higher magnification, showing individual ADK-positive cells. **(D)** CA3 region (see inset in **B**) from an *Adktm1*^{-/-}-Tg(*UbiAdk*) mouse showing the ubiquitous expression of transgenic ADK. Note the strong ADK immunoreactivity in pyramidal cell neurons in the *Adktm1*^{-/-}-Tg(*UbiAdk*) section, which is not apparent in the wild-type section. **(E)** Dentate gyrus of a wild-type hippocampus (see inset in **A**). Note the absence of distinct ADK staining in the granular cell layer. **(F)** Dentate gyrus (see inset in **B**) from an *Adktm1*^{-/-}-Tg(*UbiAdk*) mouse. Again, note the distinct ADK immunoreactivity in granular and hilar neurons in the *Adktm1*^{-/-}-Tg(*UbiAdk*) section, which is not apparent in the wild-type section. Scale bars: **A, B**, 800 μ m; **C, D, E, F** 100 μ m.

To determine if the overexpression and novel localization of ADK results in less ambient adenosine, activation of A1Rs was investigated in *Adktm1^{-/-}-Tg(UbiAdk)* mice using *in vitro* electrophysiology. In wild-type brain, adenosine exerts presynaptic inhibition in mossy fibre CA3 synapses, primarily via activation of A1Rs (Moore *et al.*, 2003). Because of difficulties in performing correct local adenosine measurements in brain tissue and because of large variations according to the methods used (Delaney and Geiger, 1996), we chose this indirect electrophysiological approach to demonstrate ADK-dependent changes in ambient adenosine levels. We measured EPSCs in CA3 hippocampal pyramidal neurons of acute slices from wild-type and *Adktm1^{-/-}-Tg(UbiAdk)* brain in response to mossy fibre stimulation. In the presence of picrotoxin (100 μ M), seizure activity was elicited in slices from mutant animals. To prevent the picrotoxin-induced seizure activity, low concentrations of NBQX (an Ampa-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid (AMPA) receptor antagonist) were added. We attempted to evoke the same current amplitude from each cell; therefore, the mean baseline amplitudes evoked from wild type cells ($n = 4$, 51.63 ± 26.05 pA) was not significantly different from the mean baseline amplitude evoked from *Adktm1^{-/-}-Tg(UbiAdk)* cells ($n = 6$, 30.99 ± 8.33 pA; $P = 0.26$, Mann–Whitney test). The effect of DPCPX, a selective A1R antagonist, on the response was normalized according to each individual cell's baseline. Application of 200 nM DPCPX resulted in an increase in the EPSC response in wild-type mice ($258 \pm 64\%$) that was significantly different from the response in *Adktm1^{-/-}-Tg(UbiAdk)* mice ($78.8 \pm 6.5\%$; $P < 0.01$, Mann–Whitney test) (Fig. 2C), thus indicating a reduction in ambient adenosine. In contrast, the addition of 5 μ M ITU, an inhibitor of ADK, led to a reduction in the EPSC amplitude to $43 \pm 13\%$ of baseline values in slices taken from *Adktm1^{-/-}-Tg(UbiAdk)* mice ($n = 3$), thus demonstrating the restoration of adenosine-mediated inhibition after blockade of ADK. When DPCPX (200 nM) was added to the ITU-inhibited slices, the EPSC amplitude was enhanced 5-fold compared with the ITU-inhibited value ($n = 2$), showing that the ITU-induced depression of the EPSC is A1R-dependent. This set of experiments thus indicates that overexpression of transgenic ADK leads to a reduction in ambient adenosine.

Spontaneous hyperlocomotion in *Adktm1^{-/-}-Tg(UbiAdk)* mice

To investigate the functional consequences of transgenic ADK overexpression in the brain, we analysed spontaneous locomotor activity and its circadian rhythmicity on three consecutive days in *Adktm1^{-/-}-Tg(UbiAdk)* and wild-type mice. *Adktm1^{-/-}-Tg(UbiAdk)* mice displayed heightened baseline levels of locomotor activity per 24 h compared with wild-type mice [$F(1, 22) = 18.566$, $P < 0.001$] (Fig. 4A). The genotype difference was consistent over the 3-day period of recording

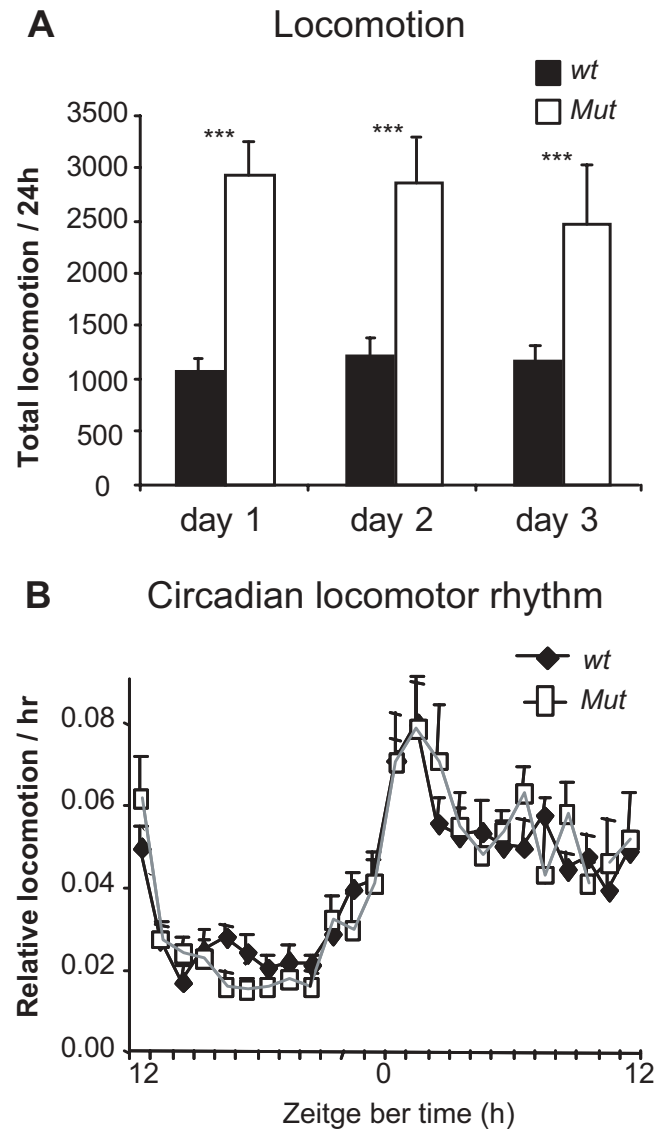


Fig. 4 Locomotion and circadian locomotor rhythmicity in *Adktm1^{-/-}-Tg(UbiAdk)*. **(A)** The total locomotion per 24 h was consistently enhanced in *Adktm1^{-/-}-Tg(UbiAdk)* mice (Mut, $n = 6$) compared with wild-type mice (wt, $n = 7$) on three consecutive days (***) ($P < 0.001$, ANOVA). **(B)** The circadian rhythmicity of locomotor activity, as expressed by relative locomotion per hour over a 12 h light/12 h dark cycle (Zeitgeber time 0 is according to offset of light), was similar in the two groups. Results are expressed as mean \pm SE.

[$F(2, 22) = 1.011$, not significant]. In addition, the circadian time-dependent changes in locomotor activity levels [$F(23, 253) = 15.904$, $P < 0.001$] were comparable in the two groups [$F(23, 253) = 0.6547$, not significant] (Fig 4B), indicating that the sleep–wake cycle, as reflected by the levels of locomotion, was not affected in these mutants. Thus, the transgene-induced ubiquitous overexpression of ADK, together with the marked elevation in brain ADK enzyme activity, probably plays a role in the hyperlocomotor phenotype of *Adktm1^{-/-}-Tg(UbiAdk)* mice.

Spontaneous and KA-induced seizure activity in *Adktm1*^{-/-}-Tg(*UbiAdk*) mice

To study the causality between ADK overexpression and seizure activity, we analysed hippocampal and cortical EEGs of untreated *Adktm1*^{-/-}-Tg(*UbiAdk*) mice. These revealed baseline activity similar to that of wild-type controls (Fig. 5A, B). However, in blocks of 6-h recordings, three of the 10 *Adktm1*^{-/-}-Tg(*UbiAdk*) mice with hippocampal electrodes displayed occasional seizure-like activity with a distinct spike pattern (4 Hz) (Fig. 5C). The duration of this spike activity ranged from 25 to 300 s. The same hippocampal spike pattern was observed in two of the 14 mutant mice equipped with both cortical and hippocampal electrodes. Another 10 of these 14 mutant mice exhibited short, high-frequency intra-ictal bursts, occurring concurrently in the hippocampus and cortex (Fig. 5E), two of which also displayed longer episodes of rhythmic, high-frequency spike activity (around 15 s) in the cortex (Fig. 5D). Each type of spike activity occurred approximately once per hour. It is important to note that none of these events was accompanied by any overt sign of convulsions or muscle twitches, thus precluding any descriptive ranking of seizures. In total, 15 out of the 24 *Adktm1*^{-/-}-Tg(*UbiAdk*) mice exhibited abnormal spiking and bursting activity in the hippocampus

and/or cortex, within a time frame of at least 6 h of recordings. None of the wild-type animals tested in this and in previous studies (Gouder et al., 2003, 2004) displayed similar EEG abnormalities.

Three of the mutants that displayed recurrent episodes of seizure-like spike activity in the hippocampus and cortex were tested for responsiveness to the ADK inhibitor ITU [3.1 mg/kg i.p. (50% of ED₅₀)] (Wiesner et al., 1999; Gouder et al., 2004). Since these mutants do not express endogenous ADK, we were able to inhibit specifically the transgene-induced ubiquitously expressed ADK. Thirty minutes after ITU injection, abnormal bursts and seizure-like spike activity were completely abolished (data not shown). Thus, the transgenic overexpression of ADK leads to occasional episodes of spontaneous seizure-like spike activity as well as abnormal bursting, which can be suppressed by inhibiting the transgenic ADK.

To investigate the consequences of constitutive ADK overexpression in an established model of temporal lobe epilepsy, we studied the EEG and histological changes following an intrahippocampal KA injection in *Adktm1*^{-/-}-Tg(*UbiAdk*) and wild-type mice. Untreated mice (*n* = 3 per genotype) served as controls. Daily analysis of intrahippocampal EEG recordings during the first 6 weeks after KA injection showed

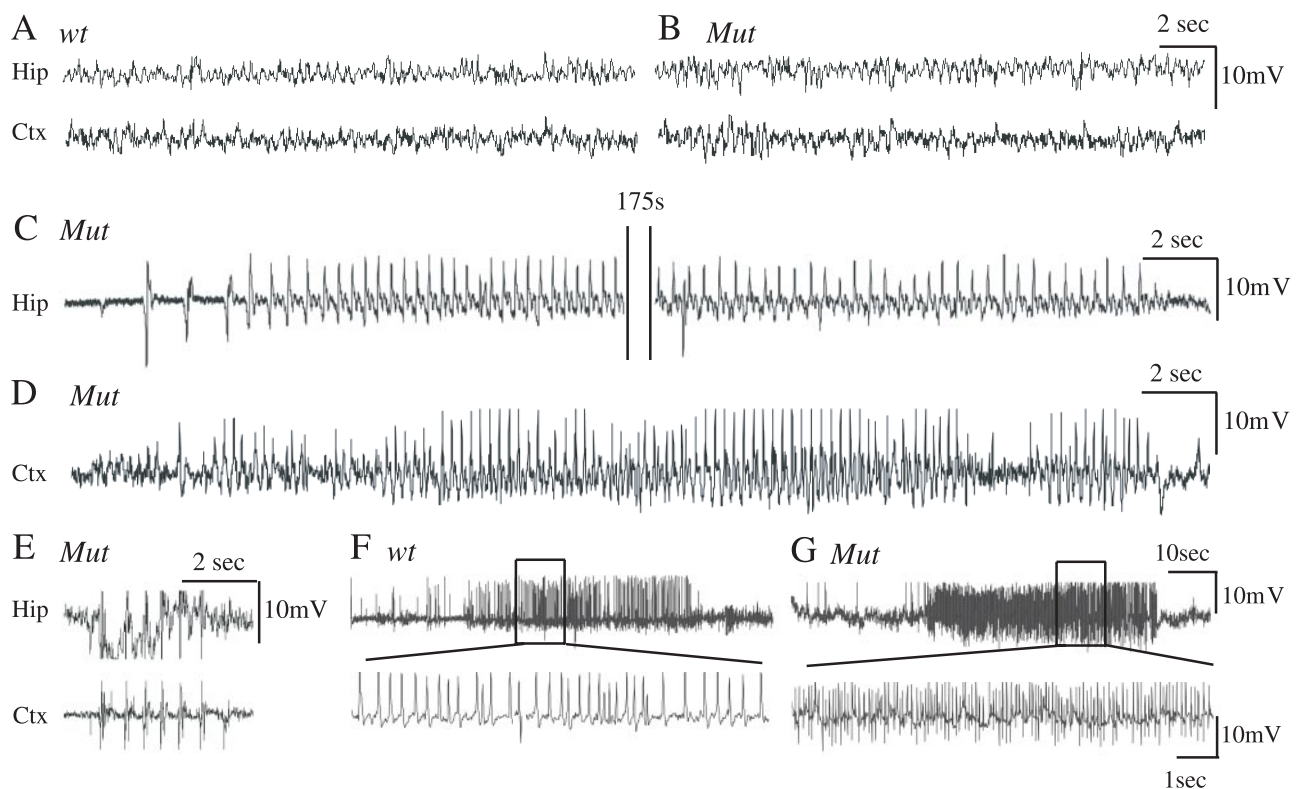


Fig. 5 Representative EEG activities from untreated and KA-injected mice. (A, B) Normal theta activity recorded in untreated wild-type (wt, A) and *Adktm1*^{-/-}-Tg(*UbiAdk*) (Mut, B) mice. (C, D, E) Representative abnormal activity occasionally observed in untreated *Adktm1*^{-/-}-Tg(*UbiAdk*) mice. (C) Seizures observed in hippocampus (Hip) and (D) cortex (Ctx). (E) Abnormal bursting observed in both hippocampus and cortex. (F, G) Seizure activity observed 4 weeks after KA injection in wild-type (wt, F) and *Adktm1*^{-/-}-Tg(*UbiAdk*) (Mut, G) mice. Note the increased intra-seizure spike frequency in KA-treated *Adktm1*^{-/-}-Tg(*UbiAdk*) mice compared with KA-treated wild-type mice.

that both *Adktm1^{-/-}-Tg(UbiAdk)* mice and wild-type mice developed recurrent electrographic seizure activity after a 2-week latent phase. It is important to note that this recurrent partial seizure activity is accompanied neither by convulsions nor by any other behavioural manifestations. Therefore, the analysis of seizure activity is limited to electrographic parameters. Recordings taken between 4 and 6 weeks after KA injection revealed both similarities and differences between the genotypes. The number of seizures per hour was similar in the two groups and the mean duration of the seizures was slightly but not significantly increased in *Adktm1^{-/-}-Tg(UbiAdk)* mice compared with wild-type seizure duration (Table 1). However, *Adktm1^{-/-}-Tg(UbiAdk)* mice were characterized by a significant increase in intra-ictal spike frequency ($P < 0.01$, compared with wild-type; Mann–Whitney test) (Fig. 5F, G; Table 1). An acute treatment with ITU (3.1 mg/kg, i.p.) was equally effective in suppressing seizure activity in the two groups (data not shown). The constitutive overexpression of ADK in *Adktm1^{-/-}-Tg(UbiAdk)* mice is thus associated with an increased intra-ictal spike frequency, once epilepsy is established.

Epileptic hippocampus in *Adktm1^{-/-}-Tg(UbiAdk)* mice does not display upregulation of astrocytic ADK

The gross histological analysis of cresyl violet-stained hippocampal sections taken 6 weeks after KA injection of *Adktm1^{-/-}-Tg(UbiAdk)* and wild-type mice ($n = 6$ animals per genotype, $n = 6$ slices per animal) revealed two characteristic features of our mouse model of KA-induced epileptogenesis: (i) in both genotypes a characteristic neuronal loss became evident in the epileptic hippocampus, notably affecting the CA1 and hilus regions; and (ii) granule cell dispersion, a characteristic hallmark of KA-induced epileptogenesis,

Table 1 EEG activity in wild-type and *Adktm1^{-/-}-Tg(UbiAdk)* 4–6 weeks after kainic acid injection

Strain	No. of seizures per hour	Mean seizure duration (s)	Mean intra-ictal spike frequency (Hz)
Wild type	23.17 ± 5.56 ($n = 6$)	44.2 ± 5.3 ($n = 6$)	4.00 ± 0.28 ($n = 6$)
<i>Adktm1^{-/-}-Tg(UbiAdk)</i>	21.00 ± 7.40 ($n = 6$)	66.7 ± 28.5 ($n = 6$)	8.50 ± 0.74** ($n = 6$)

The total number of seizures was counted during a representative hour of recording with minimal background noise. The duration of each of these seizures during the representative hour of recording was measured in seconds and averaged for each mouse. For the mean intra-ictal spike frequency, the frequency of spikes during 10 s of five representative seizures was counted and averaged for each mouse. Results are mean ± SD for seizure duration and mean ± SE for all other values (** $P < 0.01$, Mann–Whitney test).

became apparent in both genotypes (Fig. 6A, B). However, clear differences between wild-type and *Adktm1^{-/-}-Tg(UbiAdk)* mice were observed in brain sections stained for ADK immunoreactivity. The wild-type epileptic hippocampus contained a high number of ADK-positive reactive astrocytes (Fig. 6C, E). This was not the case in the epileptic hippocampus of *Adktm1^{-/-}-Tg(UbiAdk)* mice (Fig. 6D, F) which is consistent with the genetic disruption of the endogenous astrocytic ADK in these transgenic animals. In addition, in *Adktm1^{-/-}-Tg(UbiAdk)* mice, the KA-induced loss of CA1 and CA3 pyramidal cells resulted in a decrease in ADK immunoreactive neurons (Fig. 6D) compared with the number seen in untreated *Adktm1^{-/-}-Tg(UbiAdk)* hippocampus (Fig. 3B). Thus, the intrahippocampal injection of KA in *Adktm1^{-/-}-Tg(UbiAdk)* mice leads to recurrent seizure activity in the absence of endogenous astrocytic upregulation of ADK.

To determine if the development of astrogliosis is affected by the lack of KA-induced overexpression of astrocytic ADK, gliosis was examined using GFAP immunofluorescence staining. GFAP immunostaining of non-treated *Adktm1^{-/-}-Tg(UbiAdk)* and wild-type mice ($n = 6$ animals per genotype, $n = 6$ slices per animal) showed a similar distribution of astrocytes (Fig. 7A, C). In contrast, 6 weeks after KA injection astrogliosis had developed in both genotypes (Fig. 7B, D) and was essentially comparable to our previous work (Gouder *et al.*, 2004). Thus, in the absence of endogenous astrocytic ADK, *Adktm1^{-/-}-Tg(UbiAdk)* mice develop KA-induced astrogliosis. Therefore, we conclude that KA-induced astrocytic upregulation of ADK in wild-type mice is a consequence of astrogliosis.

Discussion

In the present investigation we describe the novel mouse line *Adktm1^{-/-}-Tg(UbiAdk)*, which constitutes genetic rescue of the lethal deficiency of ADK (Boison *et al.*, 2002). The rescue was achieved by introducing an *Adk* transgene (Fig. 1), which resulted in the ubiquitous expression of the short brain-specific isoform (38.7 kDa) of ADK (McNally *et al.*, 1997) (Fig. 2A). In contrast, a liver-specific transgene failed to provide genetic rescue of the lethal ADK deficiency (L. Scheurer, T. Rüllicke and D. Boison unpublished data). This finding, together with our current finding of low reconstitution of ADK in the liver of *Adktm1^{-/-}-Tg(UbiAdk)* mice (Fig. 2A), indicates that the early postnatal lethality seen in *Adktm1^{-/-}* mice may be due to a liver-independent phenomenon. Thus, the ubiquitous expression of the short isoform of ADK was sufficient and necessary to restore vital physiological functions on an ADK-deficient background.

In addition to diffuse, ubiquitous overexpression of brain ADK, which is also reflected by a marked increase in total brain ADK enzyme activity (Fig. 2B), *Adktm1^{-/-}-Tg(UbiAdk)* mice display novel ectopic neuronal expression of ADK. They lack the native ADK gene and therefore endogenous

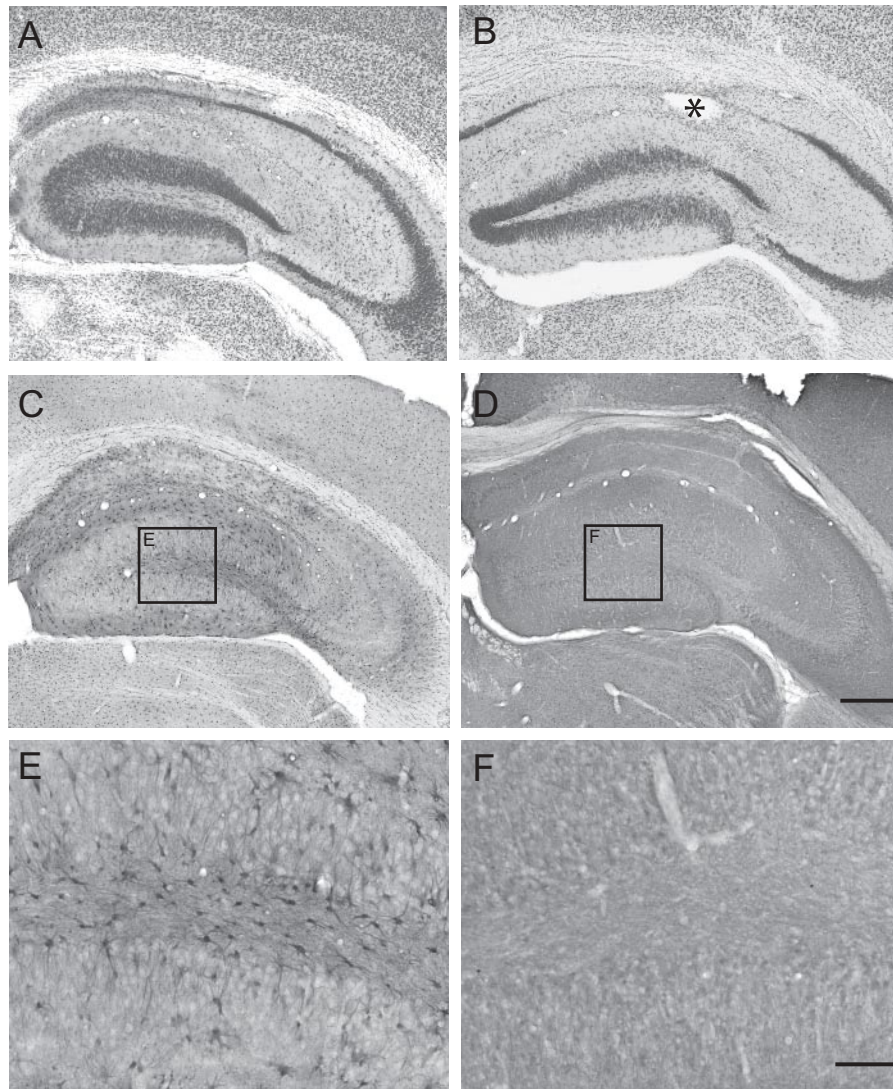


Fig. 6 Changes in hippocampal cytoarchitecture and ADK immunoreactivity 6 weeks after an intrahippocampal kainic acid injection. Representative sections taking into account variation among animals ($n = 6$ animals per genotype). (**A, B**) Cresyl violet staining of coronal sections through brains of wild-type (**A**) and *Adktm1*^{-/-}-Tg(*UbiAdk*) mice (**B**). Both genotypes developed a characteristic KA-lesion including loss of neurons in the CA1 and hilus regions and granule cell dispersion in the dentate gyrus. (**C, D**) ADK immunoreactivity of coronal sections through brains of wild-type (**C**) and *Adktm1*^{-/-}-Tg(*UbiAdk*) mice (**D**). (**E, F**) Insets in **C** and **D** at higher magnification. Note the lack of KA-induced overexpression of ADK in the mutants. The location of the bipolar intrahippocampal electrode is indicated with an asterisk (*). Scale bar: **A, B, C, D**, 500 μm ; **E, F**, 100 μm .

astrocytic ADK expression is no longer observed. Thus, *Adktm1*^{-/-}-Tg(*UbiAdk*) mice provide for the first time the opportunity to study adenosine-mediated neuromodulation in the absence of endogenous astrocytic ADK expression. In addition, the novel neuronal expression of ADK may change adenosine levels in synaptic microenvironments, thus affecting presynaptic inhibition in the hippocampus more directly. Therefore, hippocampi from *Adktm1*^{-/-}-Tg(*UbiAdk*) mice are expected to be affected by enhanced neuronal excitability.

The changes of ADK expression in *Adktm1*^{-/-}-Tg(*UbiAdk*) mice, both in cell-type specificity as well as in quantity, are likely to affect the excitability of the brain via altered adenosine levels. Brain adenosine levels, as such, are difficult to elucidate, given the fluctuations of adenosine release in

response to tissue trauma and the death of the animal (Delaney and Geiger, 1996). Therefore, we investigated the functional effects of ambient adenosine on activation of A1Rs from the mossy fibre synapses on CA3 pyramidal neurons. Several studies have shown a positive relationship between the pharmacological manipulation of extracellular adenosine and alterations in presynaptic inhibition in the hippocampus (Pak et al., 1994; Moore et al., 2003). In addition, an absence of adenosine-mediated inhibition of excitatory glutamatergic neurotransmission has been observed in adenosine A1R knockout mice (Johansson et al., 2001; Fredholm et al., 2005). Accordingly, the picrotoxin-induced seizure activity in slices from *Adktm1*^{-/-}-Tg(*UbiAdk*) mice, as well as the lack of EPSC response due to the pharmacological blockade

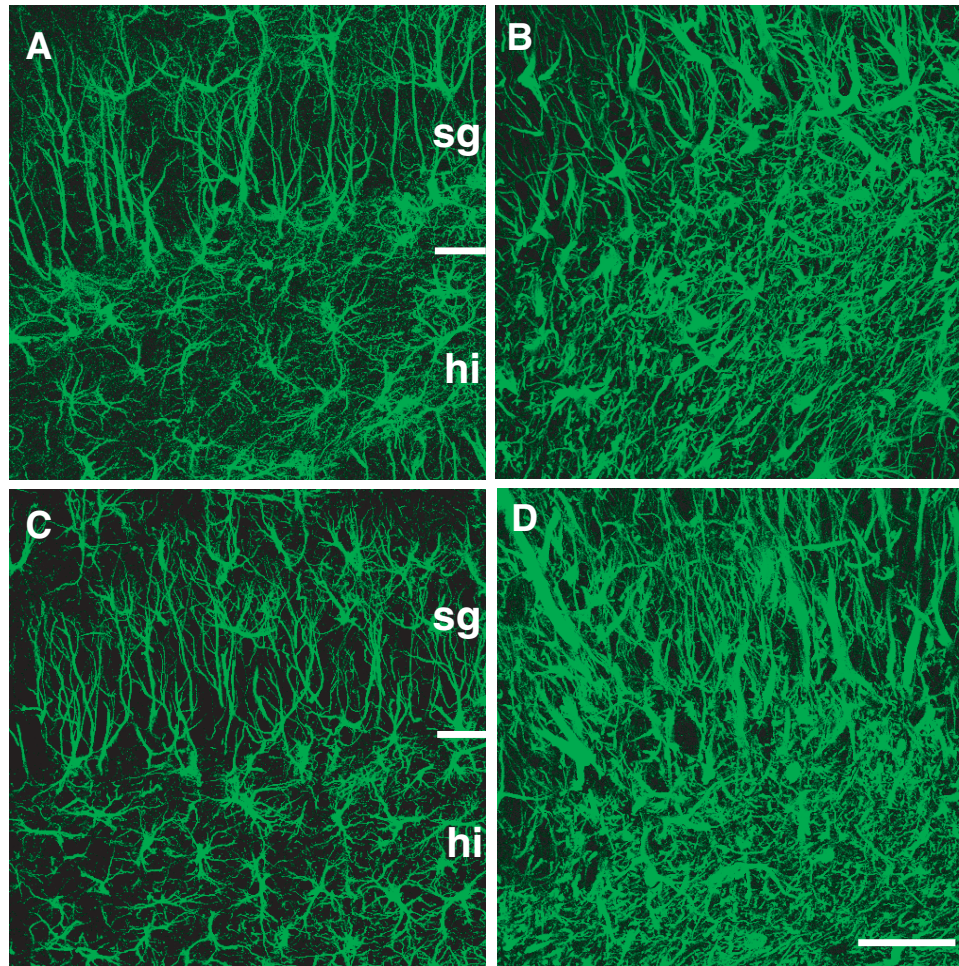


Fig. 7 GFAP immunofluorescence in representative sections from non-treated and KA-treated *Adktm1^{-/-}-Tg(UbiAdk)* and wild-type mice ($n = 6$ animals per genotype). Transverse brain sections from mice taken 6 weeks after KA injection and those from naive control animals were stained for the astrocyte marker GFAP. Optical sections were digitized at high magnification. (**A, B**) Dentate gyrus of a non-treated wild type mouse (**A**) compared with the same region of a KA-treated wild type mouse (**B**) shows the typical enlargement of astrocytic cell bodies in response to KA. (**C, D**) Dentate gyrus of a naive *Adktm1^{-/-}-Tg(UbiAdk)* mouse (**C**) compared with the same region of a KA-treated *Adktm1^{-/-}-Tg(UbiAdk)* mouse (**D**) demonstrates the development of astrogliosis in the absence of endogenous astrocytic ADK. sg = stratum granulosum; hi = hilus. Scale bar: 50 μ m.

of A1R in mossy fibre CA3 synapses from *Adktm1^{-/-}-Tg(UbiAdk)* mice, is consistent with a reduction in hippocampal adenosine levels. Concordantly, A1R-dependent adenosine-mediated inhibition of the EPSC amplitude could be restored by inhibition of ADK. The overexpression of ADK is ubiquitous, affecting all brain regions in *Adktm1^{-/-}-Tg(UbiAdk)* mice; therefore, they probably display an overall decrease in adenosinergic tone due to the increased ADK enzyme activity. The resulting lack of adenosinergic inhibition throughout the brain is manifested behaviourally by a marked increase in locomotor activity baselines without alteration of the circadian locomotor rhythm (Fig. 4). This observation is in line with the well-documented motor stimulant properties of adenosine receptor antagonists (Popoli *et al.*, 1998; Svenningsson *et al.*, 1999).

A 1.8-fold hippocampal overexpression of ADK, confined to hypertrophied astrocytes, has been associated with astrogliosis and recurrent seizure activity in the mouse kainate

model of temporal lobe epilepsy (Gouder *et al.*, 2004). Because *Adktm1^{-/-}-Tg(UbiAdk)* mice display constitutive expression of ADK in both neurons and astrocytes in the absence of endogenous astrocytic ADK, these mutants provide an ideal tool to study the causal relationship between astrocytic overexpression of ADK, astrogliosis and seizure activity. The transgene-induced overexpression of ADK in *Adktm1^{-/-}-Tg(UbiAdk)* mice correlates with an increase in ADK activity (2.2-fold compared with control, Fig. 2B) comparable to the increase in ADK activity in epileptic, KA-treated wild-type mice. This increase in ADK activity resulted in recurrent episodes of high frequency spike activity in about two-thirds of untreated *Adktm1^{-/-}-Tg(UbiAdk)* mice (Fig. 5C–E). This electrographic spike activity mimicked closely the electrographic seizures recorded in the mouse kainate model of epilepsy (Fig. 5F) and was not accompanied by convulsions or muscle twitches, thus precluding any descriptive ranking of seizures. However, treatment of

Adktm1^{-/-}-Tg(UbiAdk) mice displaying seizure-like spike activity with ITU allowed the inhibition of the transgenic ADK, which effectively suppressed the abnormal spiking activity. This shows that the overexpression of ADK is responsible for the electrographic seizure-like activity observed in mutant mice, and when adenosine levels are reconstituted by inhibition of ADK the brain resumes a normal firing pattern. These data suggest that overexpression of ADK, and the subsequent decrease in adenosinergic tone, is a cause of seizure activity. This assertion is supported by other studies which showed that decreased protective adenosine levels are likely to result in a lower threshold for seizure induction (Etherington and Frenguelli, 2004). Also, adenosine receptor antagonists can elicit seizures in the hippocampal CA3 area (Dunwiddie, 1999), prolong epileptic seizures (Dragunow and Robertson, 1987) or convert recurrent seizure activity into status epilepticus (Young and Dragunow, 1994).

In addition, the cell-type-specific expression pattern of ADK and its endogenous gene regulation may play a crucial role in astrogliosis. It is important to note that, in contrast to endogenous ADK, which is upregulated in parallel to astrogliosis and seizure activity, the transgenic ADK appears to be constitutively expressed at high levels; 6 weeks after intrahippocampal KA injection, *Adktm1^{-/-}-Tg(UbiAdk)* mice showed no sign of epilepsy-associated upregulation of ADK (Fig. 6 D, F). We have recently proposed that the gradual increase in astrocytic ADK expression in KA-treated wild-type mice may contribute to epileptogenesis (Gouder et al., 2004). However, from this previous study, we could not determine whether overexpression of ADK in the epileptic hippocampus was a cause or a consequence of astrogliosis or seizure activity. The occurrence of astrogliosis in the absence of endogenous astrocytic ADK, as observed in KA-injected *Adktm1^{-/-}-Tg(UbiAdk)* mice (Figs 5 and 6), clearly demonstrates that the overexpression of ADK in the epileptic hippocampus is a consequence of astrogliosis.

The reactive overexpression of ADK after KA-induced gliosis and seizure activity might be explained as a compensatory mechanism. The initial injection of KA into the hippocampus would cause immediate excitotoxic neuronal death and status epilepticus, triggering endogenous protective mechanisms, including the release of adenosine (During and Spencer, 1992). Continued cell death, astrogliosis and reorganization of the hippocampus during development of chronic epilepsy occur while ADK expression is increasing to abnormally high levels (Gouder et al., 2004). This increase in ADK expression may also provide a salvage route for the adenine nucleotides needed to sustain nucleic acid synthesis during reorganization of the hippocampus. Since it is known that adenosine accumulates during seizure activity, it is conceivable that the chronic release of adenosine during the epileptic state continues to reinforce the overexpression of ADK in astroglial tissue. The effect of overexpressed ADK may be further aggravated by decreased activity of A1Rs, as has been demonstrated in the hippocampus of kindled rats (Rebola et al., 2003). Thus, a vicious circle can be envisaged in

that, through overexpression of ADK and reduction of A1Rs, the threshold for seizure induction during interictal periods is reduced, thereby contributing to the progressive course of chronic epilepsy. This hypothesis is supported by our finding that KA-injected *Adktm1^{-/-}-Tg(UbiAdk)* mice display a significantly increased intra-seizure frequency of discharges as well as a trend towards longer seizure duration compared with KA-injected wild-type mice. These findings indicate that, once epilepsy is fully established, the constitutive transgenic overexpression of ADK and the novel neuronal expression of the enzyme may further increase seizure activity. On the other hand, the number of seizures per hour in KA-injected *Adktm1^{-/-}-Tg(UbiAdk)* mice was not changed compared with controls. This finding could indicate that the lack of astrocytic upregulation of ADK in KA-injected mutants prevents a further decline in protective adenosine levels, thus protecting the animals from an increase in seizure numbers.

In summary, we have shown that overexpression of brain ADK on its own can lead to seizure activity. In addition, in the KA model of temporal lobe epilepsy, overexpression of ADK is a consequence of astrogliosis. Thus, astrogliosis-induced overexpression of ADK in the KA model most likely leads to the perpetuation of chronic seizure activity.

This study clearly demonstrates that dysregulation of the adenosinergic system via a change in the expression pattern of ADK is linked to seizure activity. Therefore, epilepsy-associated overexpression of ADK provides a rationale for therapeutic intervention. The notion that an imbalance of adenosinergic signalling contributes to seizure activity and could be a therapeutic target is supported by the fact that drugs which augment the adenosine response, e.g. ADK inhibitors (Wiesner et al., 1999; Kowaluk and Jarvis, 2000; Gouder et al., 2004; McGaraughty et al., 2005), adenosine receptor agonists (von Lubitz et al., 1993; De Sarro et al., 1999; Gouder et al., 2003) and intraventricular adenosine (Boison et al., 1999), suppress seizures. Here, the adenosinergic imbalance in *Adktm1^{-/-}-Tg(UbiAdk)* mice, which resulted in spontaneous seizures and exacerbation of KA-induced seizures, was able to be treated by ADK inhibition, providing further evidence that adenosinergic therapy would be beneficial for the treatment of epilepsy. Because of peripheral side-effects of adenosine (Dunwiddie, 1999), effective strategies that enhance the protective effects of adenosine in an area of adenosinergic dysfunction (i.e. a seizure focus) may require a novel approach, such as the local delivery of adenosine by cellular implants. This approach has proven successful in the rat kindling model of epilepsy (Huber et al., 2001). Thus, future work should focus on therapeutic cellular implants releasing adenosine in the vicinity of an epileptic lesion, where ADK is overexpressed. By locally reconstituting the inhibitory adenosinergic tone, it is expected that seizure activity would be suppressed.

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