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10 Methylxanthines, seizures and excitotoxicity

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Abstract

Clinical evidence, in particular the wide use of theophylline as bronchodilator, suggests that methylxanthines can cause seizures in patients without known underlying epilepsy. Theophylline is also known to be an added risk factor for seizure exacerbation in patients with epilepsy. The proconvulsant activity of methylxanthines can best be explained by antagonizing the brain's own anticonvulsant adenosine. Recent evidence suggests that adenosine dysfunction is a pathological hallmark of epilepsy contributing to seizure generation and seizure spread. Conversely, adenosine augmentation therapies are effective in seizure suppression and prevention, whereas adenosine receptor antagonists such as methylxanthines generally exacerbate seizures. The impact of the methylxanthines caffeine and theophylline on seizures and excitotoxicity depends on timing, dose, and acute *versus* chronic use. New findings suggest a role of free radicals in theophylline-induced seizures and adenosine-independent mechanisms for seizure generation have been proposed.

A. Introduction

Seizures, ranging from altered states of consciousness to clonic and/or tonic convulsions, are commonly encountered in patients who do not have epilepsy (Delanty et al. 1998). Among other potential triggers, such non-epileptic seizures can be provoked by medication or medication withdrawal. Within this context, seizures are potentially severe or fatal complications of theophylline therapy. Theophylline can trigger seizures in patients without known underlying epilepsy and is an added risk factor for seizure exacerbation in patients with epilepsy. Most of these seizures result from toxic theophylline serum concentrations and are difficult to control. Nevertheless, clinical diagnosis and management of theophylline-induced seizures are underappreciated compared to other drug toxicities. Despite a long clinical history of theophylline-induced seizures, relatively little is known about the underlying molecular mechanisms that contribute to methylxanthine-induced seizure generation. Knowledge gained from patient data, but most notably from animal or *in vitro* studies aimed at elucidating the role of endogenous adenosine in seizure control contributes to our current understanding how methylxanthines influence the excitability of the brain.

B. Clinical findings

Anecdotally, caffeinated beverages are "known" to lower seizure thresholds in patients with epilepsy and the avoidance of excessive caffeine has been recommended in patients with epilepsy (Kaufman and Sachdeo 2003). However, due to the lack of well-designed, randomized, and placebo-controlled clinical trials, this concept has been challenged (Asadi-Pooya et al. 2008). Clinical findings in support of a proconvulsive role of methylxanthines are largely based on theophylline (or aminophylline, a mixture of theophylline with ethylenediamine that is 20 times more soluble than theophylline alone) which, clinically, is widely used to manage bronchospasms in reversible airway obstruction associated with stable asthma and chronic bronchitis (Barnes 2005; Van Dellen 1979). In addition, aminophylline is indicated in asystolic cardiac arrest and periarrest bradycardia refractory to atropine, whereas caffeine is used to treat diabetic cardiac autonomic neuropathy (Duby et al. 2004). Theophylline

has a narrow therapeutic window with an optimal plasma concentration of 10–20 mg l^{-1} (55–110mmol l^{-1}). Above this concentration, side effects such as arrhythmias and convulsions may occur, especially when given rapidly by intravenous injection (Nolan et al. 2005). Theophylline-associated seizures (TAS) or status epilepticus are considered a neurological emergency with potentially fatal outcome (Nakada et al. 1983). These seizures – largely focal onset generalized motor seizures – tend to be the only sign of theophylline toxicity, and can occur in neurologically intact patients (Aminoff and Simon 1980; Nakada et al. 1983). Remarkably, anticonvulsant therapy is ineffective in controlling these seizures, which often progress to status epilepticus and become intractable (Nakada et al. 1983; Yoshikawa 2007). Thus, in a recent clinical study the usual first-line treatment of diazepam was found to be more likely ineffective in TAS cases compared to non-TAS cases (Yoshikawa 2007); the failure of diazepam to stop those seizures might be based on interactions of theophylline with benzodiazepines (see below) (Yoshikawa 2007).

Interestingly, TAS is most common in pediatric patients under 5 years of age (Korematsu et al. 2008; Yoshikawa 2007), which can be considered to be naïve to theophylline or caffeine. In a recent study of eight pediatric TAS cases without underlying epilepsy, all had fever at the onset of TAS (> 38 °C), and six out of eight had a family history of febrile seizures and/or idiopathic epilepsy (Korematsu et al. 2008). The authors of this study concluded that in infants with an idiopathic reduced seizure threshold and fever, theophylline administration might possibly be sufficient to trigger a seizure. Apart from TAS discussed here, methylxanthine-induced seizures have also been described after the consumption of caffeinated energy drinks (Iyadurai and Chung 2007), and theophylline, caffeine, and aminophylline are used clinically to prolong seizure durations in electroconvulsive therapy for major depression (Stern et al. 1999). The potential risks associated with theophylline therapy are now well recognized. Due to concerns of CNS stimulant effects, theophylline use in patients with insomnia has been included in the 2002 Criteria for Potentially Inappropriate Medication Use in Older Adults (Fick et al. 2003).

Pharmacokinetic drug interactions of methylxanthines need also to be considered. Theophylline is largely metabolized by the hepatic enzyme CYP1A2, which is induced not only by a variety of antibiotics (Gillum et al. 1993) but also by the commonly used enzyme-inducing antiepileptic drugs, phenobarbital, phenytoin, carbamazepine, and primidone, and might require an increase in the therapeutic dose of theophylline (Patsalos et al. 2002; Spina et al. 1996). In view of the potential seizure-inducing effects of theophylline, the use of theophylline in patients with epilepsy is now limited despite the fact that second generation antiepileptic drugs do not interfere with the pharmacokinetics of theophylline (Patsalos et al. 2002). Of note, caffeine comedication in combination with phenobarbital during the first trimester of pregnancy leads to a significant increase in congenital malformations in offspring (Samren et al. 1999).

C. Experimental findings

The preconvulsive potential of methylxanthines has been corroborated in countless animal studies that reach back to more than 35 years (Roussinov et al. 1974). Early studies suggest slight differences in the convulsive role of methylxanthines: intraperitoneal administration of caffeine produced immediate excitation and seizures followed by an encephalopathy, whereas progression from encephalopathy to seizures was observed following aminophylline administration (Chu 1981). The proconvulsant and convulsant effects of methylxanthines are generally depend on dose and mode of application: Aminophylline at 100 mg/kg is known to increase the susceptibility of rats to pilocarpine or pentylenetetrazol induced seizures (Chakrabarti et al. 1997; Turski et al. 1989), whereas higher doses of aminophylline (250 mg/kg) lead to seizures and death in rats (Chakrabarti et al. 1997). These detrimental effects of

high doses of aminophylline could be avoided by using equivalent doses of theophylline in preparations of acepifylline (theophylline ethanoate of piperazine) (Chakrabarti et al. 1997). Aminophylline-induced seizures directly depended on cerebrospinal fluid concentrations of theophylline and were not influenced by metabolites of theophylline (Ramzan and Levy 1986). In several experimental combinations it was shown that methylxanthines reduce or abolish the anticonvulsant activity of several antiepileptic drugs (Kulkarni et al. 1991). In contrast, the anticonvulsant effectiveness of felbamate was only affected at higher doses of aminophylline and caffeine (Gasior et al. 1998) and aminophylline did not alter the ability of gabapentin to protect mice against seizures induced by electroconvulsive shock (Luszczki et al. 2007). The concept that methylxanthines can exacerbate seizures in epilepsy has recently been challenged by Loscher, arguing that CNS stimulants exert (pro)convulsant activity only at supratherapeutic doses (Loscher 2009).

Whereas methylxanthine induced seizures are refractory to diazepam in patients, it is important to point out that levetiracetam and several other antiepileptic drugs that do not act via activation of $GABA_A$ receptors are highly effective in suppressing caffeine-induced seizures in mice (Klitgaard et al. 1998). Astemizole, a novel histamine H_1 receptor antagonist, at a dose of 2 mg/kg increased the threshold for aminophylline-induced seizures (Swiader et al. 2005), an interesting observation since these drugs are usually combined during the treatment of asthma.

Pharmacokinetic and pharmacodynamic drug interactions have also been studied in animal models. Of note are interactions of the fluoroquinolone class of antibacterials with theophylline. In one study, chronic pretreatment of rats with the fluoroquinolone pefloxacine was shown to exacerbate aminophylline-induced seizures without altering brain concentrations of theophylline (Imperatore et al. 1997). Likewise certain environmental toxins, such as toluene, were shown to reduce thresholds for methylxanthine induced seizures (Chan and Chen 2003).

D. Adenosine, seizures, and excitotoxicity

Several potential mechanisms have been discussed that could explain the proconvulsive role of acute theophylline (Yoshikawa 2007): *(i)* general decrease of seizure thresholds; *(ii)* inhibition of adenosine A_1 receptors (A_1Rs) that normally suppress seizures by blocking the release of excitatory amino acids; *(iii)* inhibition of cerebral blood flow via adenosine antagonism (Puiroud et al. 1988); *(iv)* inhibition of 5'-nucleotidase and decrease in endogenous adenosine production; *(v)* inhibition of pyridoxal kinase, an enzyme needed for the synthesis of GABA; *(vi)* increase in cyclic GMP that is involved in maintaining the epileptic discharge; and *(vii)* a presumed direct inhibition of the $GABA_A$ receptor (Sugimoto et al. 2001), although interactions between $GABA_A$ receptors and the adenosine system might also be involved (Bonfiglio and Dasta 1991; Phillis 1979). Overall, it appears that theophylline does not trigger seizures as such, but rather potentiates pre-existing brain hyperexcitability, a mechanism consistent with the role of A_1Rs in preventing seizure spread and in mediating seizure arrest (Fedele et al. 2006; Lado and Moshe 2008; Young and Dragunow 1994). Given the dominant role of the adenosine system in seizure control within the context of theophylline toxicity, the following sections focus on the role of adenosine in epilepsy.

1. Adenosine deficiency and seizure generation

The role of adenosine as an endogenous regulator of hippocampal excitability was first recognized by Dunwiddie almost 30 years ago (Dunwiddie 1980). In a subsequent study it was shown that theophylline and other alkylxanthines antagonized electrophysiological responses to adenosine and adenosine-stimulated cyclic AMP formation, indicating that alkylxanthines increase hippocampal excitability by antagonizing the actions of adenosine (Dunwiddie et al. 1981). Thus, several adenosine receptor agonists that activate the A_1R were shown to suppress

seizures in a variety of models, albeit accompanied by sedative and hypothermic side effects (Dunwiddie and Worth 1982). Endogenous adenosine is a potent regulator of hippocampal activity and was recently shown to control hippocampal sharp waves in CA3 via activation of A_1Rs (Wu et al. 2009). It is now well recognized that adenosine is an endogenous anticonvulsant and regulator of brain activity (Boison 2005; Dunwiddie and Masino 2001; Fredholm et al. 2005a; Fredholm et al. 2005b; Ribeiro et al. 2002). The anticonvulsant activity of adenosine is largely mediated by activation of A_1Rs, since A_1R knockout mice experience spontaneous seizures (Li et al. 2007a) and are highly susceptible to seizure spread (Fedele et al. 2006). Conversely, A_1R agonists are highly effective in the suppression of seizures (Benarroch 2008; Fredholm 2003; Jacobson and Gao 2006), and have been demonstrated to suppress seizures that are resistant to conventional antiepileptic drugs (Gouder et al. 2003). Decreased extracellular adenosine levels and reduced A_1R activation as a consequence of kindling or caused by hypercapnia in a hippocampal slice preparation provide a plausible mechanisms for seizure generation (Dulla et al. 2005; Rebola et al. 2003).

In adult brain synaptic levels of adenosine are largely regulated by an astrocyte-based adenosine cycle (Boison 2008). Under physiological conditions synaptic adenosine is largely derived from vesicular release of ATP from astrocytes followed by extracellular cleavage into adenosine (Pascual et al. 2005), although astrocytic release of ATP via hemichannels has been demonstrated (Iglesias et al. 2009; Kang et al. 2008). In adult brain adenosine is rapidly phosphorylated into 5′-adenosine monophosphate by the astrocyte-based enzyme adenosine kinase (ADK; EC 2.7.1.20) (Boison 2006, 2008). In contrast to conventional neurotransmitters, such as glutamate or glycine, there is no transporter-based regulatory mechanism to terminate the synaptic activity of adenosine. Due to the presence of two types of equilibrative nucleoside transporters in the astrocyte membrane (Baldwin et al. 2004), intracellular ADK is able to fulfill the role of a metabolic reuptake system for adenosine (Boison 2008). Based on its low K_M for adenosine, ADK is the key regulator for ambient concentrations of adenosine (Boison 2006; Etherington et al. 2009; Lloyd and Fredholm 1995).

ADK has recently been identified as a molecular link between astrogliosis and neuronal hyperexcitability in epilepsy (Li et al. 2008). Astrogliosis – a pathological hallmark of the epileptic brain – is associated with upregulation of the adenosine removing enzyme ADK (Gouder et al. 2004; Li et al. 2008). Remarkably, the development of spontaneous electrographic seizures coincides both spatially (Li et al. 2008), as well as temporally (Li et al. 2007a) with astrogliosis and upregulated ADK. Uncoupling of astrogliosis from epileptogenesis in ADK-transgenic mice (Adk-tg) (Li et al. 2009) has demonstrated that overexpression of ADK, rather than astrogliosis *per se*, can be the cause for seizures. In line with these findings, Adk-tg mice express spontaneous recurrent electrographic seizures (Li et al. 2007a). Conversely, therapeutic augmentation of the adenosine system is very effective in suppressing seizures (Boison 2009). Together, these findings demonstrate that adenosine-deficiency and therefore deficient activation of A_1Rs can be a direct cause for seizures. This conclusion supports the notion that methylxanthines have proconvulsant activity due to antagonizing the function of the endogenous anticonvulsant adenosine.

2. Adenosine deficiency and excitotoxicity

Adenosine, acting via A_1Rs, is not only an endogenous anticonvulsant of the brain, but also a powerful neuroprotectant (Cunha 2005; Fredholm 1997). Thus, in addition to a proconvulsant role of A_1R deficiency or increased adenosine clearance (overexpression of ADK), these conditions lead to increased vulnerability to excitotoxic injury. Consequently, A_1R knockout mice are highly susceptible to seizure-induced (Fedele et al. 2006) or traumatic (Kochanek et al. 2006) brain injury and A_1R knockout mice experience highly aggravated neuronal cell loss after status epilepticus (Li et al. 2007a).

Pharmacological studies in a model of oxygen glucose deprivation (OGD) suggest that whereas A_1Rs desensitize after prolonged agonist exposure, A_{2A}R mediated facilitation of glutamate release by endogenous adenosine remains fully operational under long-term OGD (Sperlagh et al. 2007). Thus, the inhibition of A_{2A}Rs might be a more effective approach to attenuate glutamatergic excitotoxicity than the stimulation of A_1Rs (Cunha 2005). Consequently, A_{2A}R antagonists are actively investigated clinically for their neuroprotective potential (Chase et al. 2003; Hauser et al. 2003).

3. Adenosine-based therapeutic approaches

Given the prominent role of adenosine as endogenous anticonvulsant and neuroprotectant, adenosine augmentation therapies (AATs) are highly effective in preventing seizures (Boison 2009). Pharmacologically, seizures can be suppressed by A_1R agonists (Benarroch 2008) or by ADK inhibitors (McGaraughty et al. 2005), however systemic augmentation of the adenosine system is associated with significant side effects, including the suppression of cardiac function and depression of blood pressure, and therefore not a therapeutic option (Dunwiddie and Masino 2001). Alternatives are focal AATs to avoid systemic side effects and to restore adenosinergic signalling within a localized area of adenosine dysfunction, which can be equated with an epileptogenic focus (Li et al. 2008). Strategies that have been explored include the implantation of adenosine-releasing silk-based polymers into the infrahippocampal fissure in kindled rats. Rats treated with these polymers were protected both from established seizures, as well as from developing epilepsy (Szybala et al. 2009). Likewise, rats with focal implants of adenosine-releasing encapsulated fibroblasts or ADK-deficient stem cells were protected from kindled seizures or kindling development, respectively (Huber et al. 2001; Li et al. 2007b). Stem-cell derived adenosine-releasing implants that were placed into the infrahippocampal fissure in mice were shown to suppress acute chemoconvulsant induced seizures with associated injury (Ren et al. 2007), and to suppress epilepsy-development and spontaneous seizure expression in a model of CA3-selective focal epileptogenesis (Li et al. 2008). Together, these data demonstrate that focal re-constitution of adenosine signalling within an area of acquired adenosine dysfunction (i.e. within an epileptogenic focus) constitutes a powerful approach to suppress seizures.

E. Methylxanthines, seizures, and excitotoxicity

The above paragraphs suggest that methylxanthines – via antagonizing adenosine's anticonvulsant and neuroprotective actions (Fredholm et al. 1999; Nehlig et al. 1992) – are proconvulsants that aggravate excitotoxicity. There are, however, additional interactions that need to be considered: the influence of methylxanthines on seizures and excitotoxicity is context- and receptor-dependent, and appears to be influenced by pathways not related to adenosine.

1. Acute versus chronic caffeine

Whereas the proconvulsive activity of acute methylxanthines has long been recognized (see above), the chronic dosing of caffeine has different effects. Thus, caffeine administered at a dose of 60–70 mg/kg per day in mice over a period of two weeks (resulting in plasma levels of caffeine in the range of 6 to 14 μM, corresponding to chronic caffeine use in humans) reduced N-methyl-D-aspartate (NMDA)-, bicuculline-, and pentylenetetrazol- induced seizures in mice in the absence of changes in A_1R, A_{2A}Rs, or $GABA_A$Rs (Georgiev et al. 1993; Johansson et al. 1996). The effect was due to the combined effects of theophylline, to which caffeine is metabolized in brain, and caffeine itself but could not be ascribed to changes in A_1 and A_{2A} adenosine or $GABA_A$ receptors (Johansson et al. 1996). In contrast, higher plasma concentrations of caffeine (100 μM) after chronic dosage for 12 days resulted in increased A_1R densities, whereas mRNA levels or A_{2A}Rs were not affected (Johannson et al. 1993).

Remarkably, chronic caffeine administration in rats (40 mg/kg, twice daily for seven days) increased the thresholds for subsequent theophylline-induced seizures (Zhi and Levy 1990). This phenomenon of effect inversion might be an explanation why children (which are considered to be caffeine-naïve) appear to be more sensitive to TAS-induced seizures (see above). Effect inversion of chronic adenosine receptor antagonists has also been described within the context of ischemic excitotoxicity (de Mendonca et al. 2000). Whereas acute methylxanthines generally aggravate ischemic injury, the chronic use of caffeine or of the A_1R-selective antagonist DPCPX protects the brain from ischemic injury (de Mendonca et al. 2000). The phenomenon of effect inversion of acute *versus* chronic caffeine has intensively been studied and has been explained by antagonism of an endogenous agonist that downregulates A_1Rs without affecting gene transcription (Jacobson et al. 1996). Evidence for effect inversion by caffeine or adenosine receptor ligands has been obtained through changes in physiological outcome parameters such as susceptibility to seizures or to seizure- and ischemia- induced neuronal cell death (Jacobson et al. 1996). Despite these clear physiological changes the molecular mechanisms behind this phenomenon appear to be more complex since upregulation of A_1Rs as a consequence of chronic caffeine was not always observed (Georgiev et al. 1993; Johansson et al. 1996). Later studies have ruled out upregulation of A_1Rs as a consequence of the long-term use of caffeine or theophylline in reasonably normal doses, indicating that upregulation of A_1Rs is triggered only by excessively high or toxic doses of methylxanthines (Svenningsson et al. 1999). Thus, selected doses and durations of exposure and withdrawal, as well as A_{2A}R mediated effects (see below) might play an important role.

In a recent study a single dose of acute caffeine (40 mg/kg i.p.) given *after* the onset of seizures in a new mouse model of sudden unexplained death in epilepsy (SUDEP) significantly increased the survival time from 24 to 55 minutes (Shen et al. 2009). This protective effect of acute caffeine can best be explained by antagonizing a seizure-induced surge of adenosine, which had experimentally been exacerbated by pharmacological disruption of adenosine clearance. In this model of SUDEP excessive seizure-induced concentrations of adenosine are thought to induce cardiac and respiratory failure by overstimulation of brainstem adenosine receptors, an effect that can be ameliorated by caffeine-induced blockade of these receptors (Shen et al. 2009).

2. Caffeine: A_1 and A_{2A} receptor-mediated actions

Whereas the anticonvulsant role of A_1Rs is well established, newer findings suggest that A_{2A}Rs play an important role in modulating the susceptibility to seizures. Thus, A_{2A}R knockout mice are partially resistant to limbic seizures induced by chomoconvulsants or to seizures induced by ethanol withdrawal (El Yacoubi et al. 2009; El Yacoubi et al. 2001). Interestingly, the attenuation of clonic pentylenetetrazole-induced seizures in A_{2A}R knockout mice could be mimicked in wild-type mice exposed to chronic caffeine (0.3 g/l caffeine in drinking water) during a period of 14 days prior to the seizure tests (El Yacoubi et al. 2008). However, A_{2A}R knockout mice under chronic caffeine were less protected from clonic seizures than water treated A_{2A}R KO mice, a conflicting result that was not further addressed (El Yacoubi et al. 2008). Together, these findings indicate that the protective effects of chronic caffeine might best be explained by antagonizing the A_{2A}R and thus causing a state of decreased neuronal excitability; however, these studies also indicate a proconvulsive role of chronic caffeine under conditions, during which A_{2A}R-dependent signalling is abolished.

3. $GABA_A$ receptor and phosphodiesterase (PDE) inhibition

In contrast to adenosine receptors, which are affected by caffeine plasma concentrations attainable by normal human caffeine consumption, 10 to 100 times higher concentrations are needed to inhibit $GABA_A$Rs or PDE (Fredholm et al. 1999). Therefore, a direct preconvulsive role of “physiological” doses of methylxanthines via $GABA_A$Rs or PDE appears to be unlikely.

However, caffeine can inhibit the binding of benzodiazepines to the $GABA_AR$ (Marangos et al. 1979), which might contribute to a convulsant role of high or toxic doses of methylxanthines. Inhibition of benzodiazepine binding to $GABA_ARs$ might be an explanation for the clinical findings that TAS-induced seizures are usually refractory to treatment with diazepam or other drugs that act via the $GABA_AR$ (Yoshikawa 2007).

4. Ryanodine receptor activated calcium induced calcium release

Changes in Ca^{2+} homeostasis and persistent increases in intracellular Ca^{2+} contribute to the initiation and maintenance of acquired epilepsy (DeLorenzo et al. 2005). Ryanodine receptor (RyR) mediated calcium-induced calcium release (CICR) plays a key role in regulating intracellular calcium concentrations in epileptic conditions (Pal et al. 2001). The brain RyR is a caffeine-sensitive calcium release channel and mediates the caffeine induced mobilization of Ca^{2+} from internal stores (McPherson et al. 1991; Usachev et al. 1993). The caffeine-induced release of Ca^{2+} from ryanodine-sensitive calcium stores in the neuronal endoplasmic reticulum and pathological mechanisms that potentiate this response may render neurons more vulnerable to excitotoxicity and to the expression of seizures (Chan et al. 2000; Verkhratsky 2005). Interestingly, in cultered hippocampal neurons the newer antiepileptic drug levetiracetam led to a 61% decrease in caffeine-induced peak height of intracellular Ca^{2+} (Nagarkatti et al. 2008), indicating that levetiracetam might interact with adenosine-related signsalling.

5. Free radicals in theophylline-induced seizures

A possible role of free radicals in theophylline-induced seizures was recently suggested (Gulati et al. 2005; Gulati et al. 2007; Ray et al. 2005). In the underlying studies aminophylline (50 to 250 mg/kg) dose dependently induced convulsions and mortality in rats. Seizures and mortality were attenuated by anti-oxidants (melatonin, N-acetylcysteine) and by nitric oxide (NO) synthase inhibitors (L-NAME, 7-nitroindazole). Combination of anti-oxidant and NO-reducing treatments augmented the anticonvulsant effects of single treatments. Further, the authors found increased concentrations of malondialdehyde and NO metabolites in brain homogenates of mice with aminophylline-induced seizures; accumulation of these metabolites could be attenuated by melatonin or L-NAME pretreatment. These studies suggest the contribution of free radicals in the mechanism of theophylline induced ictogenesis.

6. Inhibition of TREK-1 channels by methylxanthines

TREK-1, a member of the two-pore-domain K(+) (K(2P)) channel superfamily, plays a major role in regulating the resting membrane potential of neurons, and thus contributes to controlling neuronal excitability (Honore 2007). Using whole-cell patch-clamp recordings on human TREK-1 channel expressing CHO cells, Harinath and Sikdar demonstrated reversible inhibition of the channels, and depolarization of the membrane potential, by caffeine and theophylline in a concentration-dependent manner (Harinath and Sikdar 2005). Inhibition by caffeine and theophylline was attenuated in channels with a mutation of a protein kinase A (PKA) consensus sequence indicating involvement of the cAMP/PKA pathway. Thus, inhibition of TREK-1 dependent membrane-depolarization may contribute to seizure generation by toxic doses of caffeine or theophylline.

F. Conclusions and outlook

Although adenosine-independent mechanisms have been proposed, the majority of evidence indicates that the proconvulsant roles of methylxanthines are based on antagonism of the brain's endogenous adenosine-based seizure control system. Whereas inhibition of A_1Rs by methylxanthines can directly contribute to ictogenesis and seizure spread, under certain conditions methylxanthines can also contribute to seizure suppression. First, this can be the case after chronic drug exposure leading effect inversion and alterations in gene expression

(Svenningsson et al. 1999). Second, antagonism of A_{2A}Rs by methylxanthines may have direct anticonvulsant and neuroprotective consequences.

A detailed understanding of the convulsant role of methylxanthines is of importance since many new drugs are in clinical trials that act on adenosine receptors. For example, in recent clinical trials conducted with the A_1R antagonist rolofylline, which facilitates diuresis and preserves renal function in patients with acute heart failure (AHF) with renal impairment, the occurrence of seizures was described in some patients that were treated with higher doses of the drug (Cotter et al. 2008). This example demonstrates that caution is needed when evaluating the clinical use of new adenosine-related therapeutic agents; however, understanding the mechanisms involved in the adenosine-related control of seizure mechanisms will allow the safe use of novel drugs that act on new therapeutic principles. New approaches using gene-array based strategies might unravel novel pathways and interactions that might help explain the complex role of methylxanthines in determining the brain's susceptibility to seizures and excitotoxicity (Yu et al. 2009).

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