(New) Pharmacological Thoughts for SLC6A1

Michael A. Rogawski, M.D., Ph.D.
Departments of Neurology and Pharmacology
University of California, Davis
Sacramento, California
Karen Gale, PhD (1948-2014)
• In the vast majority of inhibitory synapses in the mammalian brain, GABA is the neurotransmitter responsible for mediating inhibition.

• Drugs that interfere with GABA transmission lead to severe overexcitation of neural circuits and, ultimately, to convulsive seizure discharge.

• However, the dynamics of CNS GABA transmission are considerably more complex.

• Blockade of GABA transmission can be convulsant or anticonvulsant depending upon the neuroanatomical site.
Area Tempestas (deep prepiriform cortex)


Substantia Nigra (midbrain)

Drugs that block GABA-mediated neurotransmission: GABA synthesis inhibitors (isoniazid, mercaptopropionic acid, AOAA) and GABA receptor antagonists (bicuculline, picrotoxin PTZ, β-carbolines, TETS)

- Specific convulsant sites: “area tempestas” (deep prepiriform cortex); inferior colliculus.
- Specific anticonvulsant sites: superior colliculus

Basic Cortical Circuit

Glutamate acting on AMPAR/NMDAR

Excitatory afferent

Feed-forward inhibition

GABA acting on GABAR

Feedback inhibition

Glutamate acting on AMPAR/NMDAR

Excitatory output

GABAR = GABA receptor
AMPAR = AMPA receptor
NMDAR = NMDA receptor
GABAergic Synapse

GABAergic Inhibitory Intereuron

Principle Neuron

IPSP

Ca^{2+}

GABA

GABA_{A} receptor

Cl^{−}

Synaptic vesicles

GAT-1

GABA

Presynaptic (axon terminal)

Postsynaptic

Action potential

Na^{+}

K^{+}
Subunit Architecture and Physiological Role of GABA\textsubscript{A} Receptors
Pharmacology of Synaptic and Extrasynaptic GABA$_A$ Receptors

Synaptic

Benzodiazepines, zolpidem active
Neurosteroids active

Extrasynaptic

Benzodiazepines, zolpidem inactive
Neurosteroids active

[Meldrum BS and Rogawski MA, Neurotherapeutics 2007;4:18-61]
Inhibitory Synapse

Inhibitors
PTZ
Picrototoxin
Bicuculline
TETS

Astrocyte

Succinic semi-aldehyde

GABA-T

GABA

Vigabatrin

Tiagabine

Glutamate

Presynaptic terminal

GAD

GABA

GABA-T

Succinic semi-aldehyde

GABA

GABA

GAT-1

GABA

Barbiturates

Benzodiazepines

Neurosteroids

Postsynaptic neuron

GABA_A receptor

CI

Extrasynaptic

Synaptic
Tiagabine Enhances Hyperpolarizing and Depolarizing GABA Synaptic Responses

Monosynaptic IPSPs evoked in presence of CNQX and CPP

Jackson MF, Esplin B, Capek R. Activity-dependent enhancement of hyperpolarizing and depolarizing γ-aminobutyric acid (GABA) synaptic responses following inhibition of GABA uptake by tiagabine. Epilepsy Res 1999
Review of toxicity and trends in the use of tiagabine as reported to US poison centers from 2000 to 2012

HA Spiller1,2, D Wiles1, JL Russell1 and MJ Casavant1,2

Abstract
Background: Tiagabine is a novel antiepileptic that acts by increasing synaptic and extracellular gamma-aminobutyric acid concentrations. Information concerning overdose of tiagabine is limited. After introduction, an increasing number of off-label uses suggested that tiagabine use would increase. However in 2005 and 2006, warnings from the Food and Drug Administration (FDA) were issued on the risk of seizures in non-epileptic and increased suicide ideation. We evaluated the temporal trends associated with these two warnings as well as clinical outcomes from tiagabine overdose.

Method: A retrospective review of all single substance tiagabine exposures in National Poison Data System (NPDS) from 2000 to 2012.

Results: A total of 2147 patients had ingested tiagabine, with a mean of 165 year−1. This was disproportionately distributed, with a steep rise leading up to 2004 (max 559 year−1) and then a significant decline (p < 0.05) between 2005 and 2006. The number of cases reported to NPDS mirrored the sales of tiagabine. Clinical effects were predominantly neurological, with the most commonly reported effects being drowsiness (27%), agitation (19%), confusion (12%), seizures (11%), and tachycardia (10%). In all, 758 patients (35%) showed a major or moderate medical outcome, with no deaths reported. A disproportionate share of the major outcomes was in the suicide attempt group (73%). The majority of patients (75%) were treated in a health-care facility (HCF).

Conclusions: The HCF usage is likely due to high rate of symptomatic patients (59%) and the large proportion of suicide attempt cases. The frequency of tiagabine cases in NPDS mirrored pharmaceutical sales, with steep declines temporally related to the 2005 FDA warning.

Keywords
Tiagabine, FDA warning, toxicity

Introduction
Tiagabine is a novel antiepileptic that acts by binding to the gamma-aminobutyric acid (GABA) uptake transporter in presynaptic neurons and glial cells resulting in increased synaptic and extracellular GABA concentrations. The increased concentration of the inhibitory neurotransmitter GABA is believed to be responsible for the antiepileptic effects. Information concerning overdose of tiagabine is limited to case reports and a single case series.1−3 These reports suggest the effects in supratherapeutic doses and overdoses that follow the GABA neurological mechanism, with lethargy, confusion, and coma. Seizures, convulsive, and nonconvulsive status epilepticus have also been reported. The epileptogenic mechanism for tiagabine is not known but suggestions have included possible mediation via GABA receptors in the thalamus and stimulation of dopamine, serotonin, or glycine receptors.6,10 An atypical

Table 1. Reported clinical effects with single substance ingestion of tiagabine.

<table>
<thead>
<tr>
<th>Clinical effect</th>
<th>N</th>
<th>Percent of total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drowsiness/lethargy</td>
<td>570</td>
<td>26.5</td>
</tr>
<tr>
<td>Agitated</td>
<td>405</td>
<td>18.9</td>
</tr>
<tr>
<td>Confusion</td>
<td>260</td>
<td>12.1</td>
</tr>
<tr>
<td><strong>Total with SZ</strong></td>
<td>226</td>
<td>10.5</td>
</tr>
<tr>
<td>SZ—single</td>
<td>89</td>
<td>4.1</td>
</tr>
<tr>
<td>SZ—multiple</td>
<td>81</td>
<td>3.8</td>
</tr>
<tr>
<td>SZ—status epileptic</td>
<td>56</td>
<td>2.6</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>213</td>
<td>9.9</td>
</tr>
<tr>
<td>Dizziness</td>
<td>106</td>
<td>4.9</td>
</tr>
<tr>
<td>Coma</td>
<td>99</td>
<td>4.6</td>
</tr>
<tr>
<td>Hypertension</td>
<td>84</td>
<td>3.9</td>
</tr>
<tr>
<td>Vomiting</td>
<td>55</td>
<td>2.6</td>
</tr>
<tr>
<td>Mydriasis</td>
<td>45</td>
<td>2.1</td>
</tr>
<tr>
<td>Slurred speech</td>
<td>39</td>
<td>1.8</td>
</tr>
<tr>
<td>Dystonia</td>
<td>35</td>
<td>1.6</td>
</tr>
<tr>
<td>Hypotension</td>
<td>23</td>
<td>1.1</td>
</tr>
<tr>
<td>Hallucinations/delusions</td>
<td>23</td>
<td>1.1</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>16</td>
<td>0.7</td>
</tr>
<tr>
<td>Muscle rigidity</td>
<td>15</td>
<td>0.7</td>
</tr>
<tr>
<td>Respiratory depression</td>
<td>26</td>
<td>1.2</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>11</td>
<td>0.5</td>
</tr>
<tr>
<td>Headache</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>Fasciculation</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td>Fever</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td>Acidosis</td>
<td>7</td>
<td>0.3</td>
</tr>
<tr>
<td>Tachypnea</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Chest pain</td>
<td>3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

SZ: seizures.

*Central Ohio Poison Center, Nationwide Children’s Hospital, Columbus, OH, USA
Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH, USA

Corresponding author:
HA Spiller, Central Ohio Poison Center, Nationwide Children’s Hospital, 700 Children’s Dr, Columbus, OH 43205, USA.
Email: haspiller5@gmail.com
CASE REPORT

Tiagabine-induced absence status in idiopathic generalized epilepsy

S. KNAKE, H. M. HAMER, U. SCHOMBURG, W. H. OERTEL & F. ROSENOW

Philipps-University, Neurologische Klinik, Marburg, Germany

Correspondence to: Dr S. Knake, Neurologische Klinik, Philipps-University, Marburg, Rudolf-Bultmann-Straße 8, 35039 Marburg, Germany

Several medications such as baclofen, amitriptyline and even antiepileptic drugs such as carbamazepine or vigabatrin are known to induce absence status epilepticus in patients with generalized epilepsies. Tiagabine (TGB) is effective in patients with focal epilepsies. However, TGB has also been reported to induce non-convulsive status epilepticus in several patients with focal epilepsies and in one patient with juvenile myoclonic epilepsy. In animal models of generalized epilepsy, TGB induces absence status with 3–5 Hz spike-wave complexes.

We describe a 32-year-old patient with absence epilepsy and primary generalized tonic-clonic seizures since 11 years of age, who developed her first absence status epilepticus while treated with 45 mg of TGB daily. Administration of lorazepam and immediate reduction in TGB dosage was followed by complete clinical and electroencephalographic remission. This case demonstrates that TGB can induce typical absence status epilepticus in a patient with primary generalized epilepsy.

Key words: absence status; status epilepticus; generalized epilepsy; seizure induction; tiagabine.

INTRODUCTION

Absence status epilepticus is defined as a generalized absence seizure lasting for more than half an hour in the context of a primary generalized epilepsy. During the time of mental clouding of differing degree, bilateral synchronous spike-wave discharges can be recorded by EEG. Of all the forms of non-convulsive status epilepticus, generalized absence status is the most commonly encountered. The incidence of absence status in patients with absence epilepsy is estimated to be between 2 and 10%.

Several antiepileptic drugs have been reported to induce absence status in patients and animal models. Tiagabine (TGB) inhibits the reuptake of γ-aminobutyric acid (GABA) into the presynaptic terminals and glia cells, thereby making more GABA available at the synapses. It increases the extracellular concentration of GABA after oral administration. In clinical trials TGB had a dose-related anticonvulsant effect in patients with focal epilepsies. However, in animal models the frequency of interictal spike-wave discharges increased in rats when treated with TGB.

In addition, TGB was also reported to induce non-convulsive status epilepticus in several patients with partial epilepsy and in one patient with juvenile myoclonic epilepsy. There have been no detailed reports of this effect of TGB in patients with absence epilepsy.

We describe a 32-year-old patient with absence epilepsy and generalized tonic-clonic seizures since the age of 11 years, in whom TGB induced her first absence status.

CASE REPORT

A 32-year-old woman was admitted with absence status epilepticus lasting for more than 6 hours.

At the age of 11 years, she began having absences and generalized tonic-clonic seizures. The diagnosis of absence epilepsy was supported by generalized spike-wave complexes during hyperventilation and a
mGAT1 KO Mouse Phenocopies Some Effects of Tiagabine

Dizziness, asthenia, somnolence (sedation), nonspecific nervousness, tremor, and ataxia

mGAT1 KO mouse is slightly more sensitive than the WT mouse to PTZ-induced seizures

PTZ (40 mg/kg, i.p.) decreased observable activity in WT and heterozygotes while causing preconvulsive states and mild seizures in mGAT1 KO mice

PTZ (70 mg/kg), all WT and heterozygotes survived with severe seizures, whereas mGAT1 KO mice showed severe seizures, and one of three died
Anticonvulsant Activity of Intravenous Muscimol in Rats

M. W. Matthews and G. P. McCarthy
Department of Biological Research, Smith Kline & French Laboratories, Philadelphia, PA 19101, U.S.A.

(Accepted 28 May 1979)

Summary—Muscimol is a potent in vivo agonist of gamma-aminobutyric acid (GABA) when tested iontophoretically and binds to GABA receptors in vitro. A study of muscimol effects on seizures induced by agents which impair GABA-mediated neurotransmission was performed in the rat. Muscimol delayed the onset of isoniazid- and picrotoxin-induced convulsions. The tonic forelimb extension component of bicuculline and pentylenetetrazole seizures was abolished by muscimol. Inhibition of forelimb extension was chosen as an endpoint for comparison of clinically effective antiepileptics with muscimol. The order of potency was diazepam > muscimol > phenobarbital > pentylenetetrazole.

Table 4. Summary of anticonvulsant testing. Comparison of muscimol with standard antiepileptic agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bicuculline</th>
<th>Convulsant Strychnine</th>
<th>Pentylenetetrazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol</td>
<td>1.0 (0.7–2.0)</td>
<td>No effect at 8 mg/kg</td>
<td>0.49 (0.32–1.5)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.32 (0.07–0.49)</td>
<td>0.82 (0.4–1.4)</td>
<td>0.09 (0.04–0.14)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>7.9 (5.1–10.7)</td>
<td>35.9 (22.2–63.8)</td>
<td>4.1 (2.7–7.4)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>9.3 (5.0–13.3)</td>
<td>136.2 (80.0–354.4)</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

Muscimol, diazepam and phenobarbital were given intravenously. Phenytoin was administered intraperitoneally. All convulsants were injected intravenously 30 min after anticonvulsant treatment. Table values are ED₅₀ (mg/kg) with 95% Fieller limits; n = 5–10/dose group.

Gamma-aminobutyric acid (GABA) is an amino acid which satisfies many of the criteria for a naturally-occurring inhibitory transmitter in the vertebrate central nervous system (Roberts, Chase and Towe, 1976; DeFeudis, 1977). Experimental evidence implicates a hypothalamic central GABA system in certain neurological disorders (Hornykiewicz, Lloyd and Davidson, 1976) including epilepsy (Meldrum, 1975). Muscimol (3-hydroxy-5-aminomethylisoxazol), a structural analog of GABA, is a potent GABA agonist at the crustacean neuromuscular junction (Wheat and Keruti, 1976) and at bicuculline-sensitive postsynaptic receptors in the mammal (Krogsgaard-Larsen, Johnston, Curtis, Game and McCulloch, 1975; Ebb, Collins and Snyder, 1977). A delay in the onset of isoniazid-induced seizures after muscimol treatment has been reported (Nalde, Guidetti and Costa, 1976). However, abolition of convulsive behavior elicited by impairment of GABAergic neurotransmission has not been demonstrated after muscimol treatment. The present authors studied the anticonvulsant activity of muscimol against seizures induced by a variety of drugs known to interfere with GABA-mediated neurotransmission and other convulsant chemicals. The anticonvulsant profile of muscimol was compared to that of several clinically effective antiepileptics.

METHODS

Male Sprague-Dawley rats (200–275 g) were used in all experiments. Animals were housed in constant temperature, humidity and light cycles. Food and water were available ad libitum.

Key words: tonic forelimb extension, picrotoxin, isoniazid, bicuculline, pentylenetetrazole, strychnine.
GAT-1 Is Present in Immature Brain But Not Functionally Active

Age-dependence of the anticonvulsant effects of the GABA uptake inhibitor tiagabine in vitro
Adriana Sabau, Christiane Frühm, Michael Pfeiffer, Jörg-Michael Breustedt, Antje Piechotta, Markus Nummerback, Dominique Engel, Uwe Heinemann, Andreas Draguhn
Johannes-Müller-Institut für Physiologie der Charité, Humboldt-Universität zu Berlin, Tucholskyweg 2, 10117 Berlin, Germany
Received 23 June 1999; accepted 26 August 1999

Abstract

Epileptic syndromes frequently start at childhood and therefore it is crucial to test new anticonvulsants at immature stages of the nervous system. We compared the effects of the $\gamma$-aminobutyric acid (GABA) uptake inhibitor tiagabine [2S-3S,4,4,4-tetrahydromethyl-2-thioline] to tiagabine [2S-3S,4,4,4-tetrahydromethyl-2-thioline][3H]-labeled 2-thioline-3-yl picoenic acid] or low-Mg$^{2+}$- induced epileptiform discharges in brain slices from rat pups (p 5-8) and juvenile animals (p 15-20). In slices from rat pups, tiagabine slightly reduced epileptiform activity in hippocampal area CA1 but had no effect in the entorhinal cortex. In juvenile rats, epileptiform discharges were unaltered in CA1 but suppressed by 80% in the entorhinal cortex. While tiagabine increases its efficacy with age, in situ hybridisation and PUR analysis show that mGluR6 coding for the neuronal GABA-transporter GAT-1 is already present at p 5. We therefore conclude that the increasing efficacy of tiagabine during ontogenesis is due to functional maturation of GABAergic synapses rather than to up-regulation of GAT-1 expression. () 1999 Elsevier Science B.V. All rights reserved.

Keywords: Epilepsy; Brain slices; Tiagabine; GABA (y-aminobutyric acid) uptake; Development; Hippocampus; Cortex, entorhinal

1. Introduction

Many epileptic syndromes in man start during childhood or adolescence and it is generally assumed that the immature brain is especially susceptible for seizure generation and for the chronification of epilepsy (Modé et al., 1985; Lothman and Bertram, 1997). Various mechanisms have been suggested to contribute to this age-dependence, including depolarising GABA$_A$ receptor-mediated potentials (Kroskent et al., 1985; Ben-Ari et al., 1988), immature regulation of K$^+$-homeostasis in the developing brain (Habicht and Heinemann, 1987), changes in the function and expression of NMDA receptors (Bradley et al., 1993) or enhanced recurrent excitatory connections (Owame et al., 1993). These developmental characteristics may be mirrored by differences in the potency or efficacy of anticonvulsant drugs. In juvenile rats, various $\gamma$-aminobutyric acid (GABA) mimetic substances show little efficacy against experimentally induced afterdischarges and tonic-clonic seizures (Veliles and Mares, 1995; Pollese et al., 1996). Microinjection of GABA$_A$ receptor agonists into the substantia nigra of rat pups exerts proconvulsant effects on flurothy-induced seizures whereas they are anticonvulsant at low doses in adults (Garratt et al., 1995). Therefore, GABAergic agents should be thoroughly tested throughout development including very immature stages. Tiagabine [2S-3S,4,4,4-tetrahydromethyl-2-thioline][3H]-labeled 2-thioline-3-yl picoenic acid] is a derivative of picoenic acid with good anticonvulsant activity both in vitro and in vivo (Faingold et al., 1994; Suzuki, 1994; Walton et al., 1994; Smith et al., 1995; Pfeiffer et al., 1996). It is effective as an ad-on therapy in complex partial seizures in adults (Garratt et al., 1994; Richells et al., 1995; Larr and Rompe, 1998) while results from children with epilepsy are not yet sufficient to allow for a comparison with established anticonvulsants (Cella and Noble, 1998). Tiagabine acts by blocking the uptake of synaptically released GABA into neurons and glia cells, thereby prolonging the postsynaptic inhibitory effect of this transmitter (Boberg et al., 1998; Mosepaert and Lambert, 1994). However, in a recent study on in vitro postsynaptic currents (PSCs) in dentate granule...
Muscimol Facilitates Seizures in Immature Brain

Latency to Flurothyl Convulsion

Bilateral nigral infusion of muscimol
Age-Related Differences in the Effects of GABA<sub>A</sub> Agonists Microinjected Into Rat Substantia Nigra: Pro- and Anticonvulsant Actions

*1D. S. Garunt, S. G. Xu, *2E. F. Sperber, and *3H. L. Moshe

Laboratory of Developmental Epilepsy, Departments of *Neurology, *Neuroscience, and *Pediatrics, Albert Einstein College of Medicine, Bronx, New York, U.S.A.

Summary: GABA<sub>A</sub>-ergic transmission in the substantia nigra pars reticulata (SNR) has an important role in the control of experimental seizures. In the fluoroethyl seizure model, SNR microinjection of the selective GABA<sub>A</sub> receptor agonist muscimol results in a biphasic dose-response curve in adults. Intermediate doses are anticonvulsant, but high doses have proconvulsant effects. Another GABA<sub>A</sub> agonist, THIP (1,4,5,6,7-hexahydro-1-methyl-1H-indazole-3-carboxylic acid), also produces anticonvulsant effects at lower doses, whereas higher doses tend to produce a proconvulsant effect. In 16-day-old rat pups, no anticonvulsant but only proconvulsant effects of muscimol occur, and at lower doses than in adults. These data suggest that the immature SNR is significantly more sensitive to the proconvulsant effects of GABA<sub>A</sub> receptor agonists than the SNR of adults. We hypothesize that the age-related differences in spinal GABA<sub>A</sub>-ergic response may be due to ontogenic changes in GABA<sub>A</sub>-sensitive neuronal circuits in the SNR. Key Words: Muscimol—THIP—Fluoroethyl—Seizures—Epilepsy—Aminotetrahydropyridine acid.

Both clinically and experimentally, it is recognized that the immature brain is at increased risk for sustaining epileptic seizures, particularly generalized seizures (1). An important goal of neurosciences research is to identify the neuronal circuitry and synaptic pharmacology underlying this ontogenetic phenomenon. Such understanding will help direct the development of more efficacious and selective age-specific therapies than those currently available.

The substantia nigra pars reticulata (SNR) has been identified as a critical site in rat brain for the anticonvulsant action of GABA<sub>A</sub>-ergic drugs (2). Since it was shown that GABA<sub>A</sub>-ergic transmission in SNR can attenuate seizures (3,4), a series of investigations (5,6) led to the hypothesis that experimental manipulations that inhibit firing in SNR neurons bilaterally can limit convulsive and subconvulsive seizure activity produced in several experimental models of epilepsy (2). However, studies suggest that the ability of SNR inhibition to control seizures may be age-dependent: drugs that are anticonvulsant when microinjected into the SNR of adult rats may be ineffective (7) or even proconvulsant (8) when injected into the SNR of rat pups.

Our laboratory has studied developmental differences in the anticonvulsant pharmacology of GABA transmission in rat SNR. We measured the susceptibility of rats to seizures induced by fluoroethyl (hexfluoroethyl ether), a volatile inhaled convulsant well suited to developmental studies (9). We commonly compare adult seizure responses with those of 16-day-old rat pups, an age at which such pups are most susceptible to seizures (1. We previously showed the effects on fluoroethyl seizures of some GABA<sub>A</sub>-ergic agents to be similar regardless of the age of the rats examined. Doses of the presynaptic GABA<sub>A</sub> agonist γ-vinyl-GABA (GVG) that increase GABA in SNR result in the suppression of fluoroethyl seizures in both adult (10) and 16-day-old rats (11). Conversely, microinjections into SNR of bicuculline, a competitive GABA<sub>A</sub> receptor antagonist (12) significantly facilitate fluoroethyl seizures in both adults (13) and pups.
GABA$_B$ Receptors

Baclofen

Inhibitory Presynaptic terminal

Ca$^{2+}$

K$^+$

GIRK

GABA$_B$

GABA$_A$

GABA$_B$
CHARACTERIZATION OF GABAERGIC SEIZURE REGULATION IN THE MIDLINE THALAMUS

J. W. MILLER and J. A. FERRARESLLI
Departments of Neurology and Neurological Surgery (Neurology) and Neuropharmacology, Washington University School of Medicine, 660 South Euclid Ave., St Louis, Missouri 63110, U.S.A.

(Accepted 29 November 1988)

Summary—This study characterized the role of GABA in the central medial intralaminar nucleus on seizures induced by pentylenetetrazol given systemically. Injections of the direct selective GABA_A agonist, piperidine-4-sulfonic acid or the indirect GABA_A agonist, flurazepam and pentobarbital, in this region depressed arousal and facilitated myoclonic and clonic seizures induced by pentylenetetrazol but only caused slight inhibition of tonic seizures. In contrast the GABA_A agonist (-)baclofen facilitated all three types of seizures. Recording after injection of piperidine-4-sulfonic acid and (-)baclofen revealed marked suppression and slowing of thalamic and cortical electrical activity. Thalamic injections of the GABA_A antagonist, bicuculline methiodide, had opposite behavioral effects, causing hyperreactivity and episodes of violent running, not accompanied by EEG discharges. When pentylenetetrazol was infused concomitantly there was marked facilitation of the tonic seizures, which occurred without preceding myoclonic or clonic seizures, or EEG spikes.

These results demonstrate that GABA-mediated neurotransmission in the central medial intralaminar nucleus can control the threshold of seizures and that GABA agonists and antagonists have opposite effects. It is suggested that the central medial intralaminar nucleus is not a site of originiation or spread of seizures, but controls seizures indirectly by regulating the excitability of other structures and that different synaptic mechanisms and anatomical connections mediate effects on different types of seizures.

Key words—pentylenetetrazol, central medial nucleus, GABA, seizure, epilepsy, thalamus, arousal.

Injection of Baclofen into Midline Thalamus

![Diagram showing the target zone in the central medial nucleus in the thalamus. For inclusion in the study, the entire blue injection site had to be confined to this zone. Abbreviations are as follows: AM—anteromedial nucleus; CeM—central medial nucleus; IAD—interanterodorsal nucleus; IAM—interanteromedial nucleus; PT—paratenial nucleus; PV—paraventricular nucleus; Re—reuniens; Rh—rhomboid nucleus.]

![Graph showing the effect of Baclofen on seizure behavior. Control: EEG and Th. Baclofen: EEG and Th.](image-url)
Baclofen augments hippocampal γ oscillations and promotes pathological discharge in the CA3 network of chronic epileptic mice in a dose-dependent manner

- Reduced inhibition of CA3 PCs and enhanced presynaptic \( \text{GABA}_B \) receptor activation
- Enhanced activation of presynaptic \( \text{GABA}_B \) receptors leads decreased \( \text{GABA} \) release and consequent disinhibition and hyperexcitability of pyramidal cells.

**Significance**

Metabotropic \( \text{GABA}_B \) receptors control synaptic transmission and excitability in neuronal circuits of the brain. Although effects of these receptors are predominantly inhibitory at both cellular and network levels, application of the agonist baclofen can promote excitability and induce seizures in patients and animal models of epilepsy. Here we demonstrate that pre-epileptic effects of baclofen are concentration dependent and result from disinhibition. Although at high doses, baclofen reduces network excitability due to its combined pre- and postsynaptic inhibitory effects in pyramidal cells, at low doses, it leads to an enhanced presynaptic suppression of the network output of a specific set of inhibitory neurons. This disinhibitory effect promotes high-frequency oscillations and the emergence of pathological discharges in the hippocampal network.


The authors declare no conflict of interest.

This article was published as an Accepted Article in advance of the print edition.

This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft) and the National Institute of Mental Health (National Institutes of Health).
GABA$_B$ Receptors

![Diagram of GABA$_B$ Receptors](image)

- $V_{\text{Rest}}$
- $-65 \text{ mV}$
- $-80 \text{ mV}$
- Activated
- Deinactivated
- T-type Ca$^{2+}$
- Ca$^{2+}$
The End
Enhanced tonic GABA\textsubscript{A} inhibition in typical absence epilepsy

David W Cope\textsuperscript{1}, Giuseppe Di Giovanni\textsuperscript{1,2}, Sarah J Fyson\textsuperscript{1}, Gergely Orbán\textsuperscript{1,3}, Adam C Errington\textsuperscript{1}, Magor L Lórinčík\textsuperscript{1}, Timothy M Gould\textsuperscript{1}, David A Carter\textsuperscript{1} & Vincenzo Crunelli\textsuperscript{1}

The cellular mechanisms underlying typical absence seizures, which characterize various idiopathic generalized epilepsies, are not fully understood, and impaired \(\gamma\)-aminobutyric acid (GABA)-ergic inhibition remains an attractive hypothesis. In contrast, we show here that extrasynaptic GABA\textsubscript{A} receptor-dependent tonic inhibition is increased in thalamocortical neurons from diverse genetic and pharmacological models of absence seizures. Increased tonic inhibition is due to compromised GABA uptake by the GABA transporter GAT-1 in the genetic models tested, and GAT-1 is crucial in governing seizuregenesis. Extrasynaptic GABA\textsubscript{A} receptors are a requirement for seizures in two of the best characterized models of absence epilepsy, and the selective activation of thalamic extrasynaptic GABA\textsubscript{A} receptors is sufficient to elicit both electrographic and behavioral correlates of seizures in normal rats. These results identify an apparently common cellular pathology in typical absence seizures that may have epileptogenetic importance and highlight potential therapeutic targets for the treatment of absence epilepsy.

Typical absence seizures characterize numerous idiopathic generalized epilepsies and are the main ictus triggers in the electroencephalogram (EEG) as bilaterally synchronous spike-and-wave discharges (SWDs) accompanied by behavioral arrest\textsuperscript{4}. Whereas absence seizures are known to arise in thalamo-cortical networks\textsuperscript{4,5}, the underlying cellular mechanisms are not fully understood. Impaired GABA\textsubscript{A}ergic inhibition remains an attractive hypothesis\textsuperscript{6,7}, and GABA\textsubscript{A} receptor (GABA\textsubscript{A}R) subunit mutations have been identified in humans with typical absence seizures, albeit as part of a complex phenotype\textsuperscript{8-10}. However, although some of these mutations compromise GABA\textsubscript{A}R function in heterologous expression systems\textsuperscript{11}, only modest changes in GABA\textsubscript{A}R inhibition have so far been identified in the thalamo-cortical network of rodents with spontaneous SWDs\textsuperscript{12,13}. Furthermore, systemic or intra-hippocampal administration of agents that promote GABA\textsubscript{A}R inhibition, including the antiepileptic drugs lamotrigine and tiagabine, inhibit or exacerbate seizures in humans and rodents\textsuperscript{14-19}. Thus, impaired rather than improved GABA\textsubscript{A}R inhibition may be a feature of absence seizures.

Activation of GABA\textsubscript{A}Rs generates two types of inhibition: the transient activation of synaptic GABA\textsubscript{A}Rs (sGABA\textsubscript{A}R) eliciting postsynaptic currents (IPSCs), or \textit{phasic} inhibition, and the activation of postsynaptic (pGABA\textsubscript{A}R) or extrasynaptic (eGABA\textsubscript{A}R) by ambient GABA causing a persistent active or tonic, current\textsuperscript{20,21}. Because in thalamocortical neurons, major players in the thalamocortical circuit during SWDs, >90% of GABA\textsubscript{A}R inhibition is tonic\textsuperscript{22-24}, we have now examined the persistent active tonic inhibition in experimental absence seizures. Our data indicate that enhanced tonic GABA\textsubscript{A}R inhibition is a common feature of diverse genetic and pharmacological models of typical absence epilepsy and may be a requirement for the appearance of absence seizures.

RESULTS Enhanced tonic GABA\textsubscript{A} current in genetic models of absence

Genetic absence epilepsy rats from Strasbourg (GAERS) are a well-established genetic model of absence epilepsy that show bilateral spontaneous SWDs and accompanying behavioral arrest from approximately postnatal day 30 (PN30). In thalamocortical neurons, tonic GABA\textsubscript{A} currents are generated by extrasynaptic receptors containing the \(\delta\) subunit\textsuperscript{22} and, in rats, \(\delta\) subunit expression is apparent only from approximately PN21 (ref. 23). Therefore, we measured tonic GABA\textsubscript{A} current amplitude from thalamocortical neurons in slices of the somatosensory ventromedial thalami of GAERS from PN4 onward and compared it to non-epileptic control (NEC) rats of the same age. We observed a significant difference in tonic current amplitude at PN4-5 (p < 0.05 for each day) (Fig. 1a,b). At PN7, however, there was an approximately twofold increase in tonic current amplitude in GAERS compared to NEC rats (p < 0.05) that was sustained in subsequent days (Fig. 1a,b) and was independent of whole-cell capacity (Supplementary Results and Supplementary Table 1). Comparison of spontaneous IPSC (sIPSC) parameters in GAERS and NEC rats at the same ages revealed no consistent differences (Supplementary Table 1). In agreement with previous data obtained from younger GAERS\textsuperscript{25}, notably, there was a significantly (p < 0.05) smaller sIPSC peak amplitude, frequency, charge transfer and total current in GAERS at PN8, but these changes were not maintained at later ages (Supplementary Table 1). These results indicate an apparent absence of tonic enhancement of sIPSCs in PN4-5 GAERS with a subsequent increase in tonic current amplitude in GAERS relative to NEC rats between PN7 and PN21.

Elevated GABA levels in GAERS rats in ventral thalamus because of reduced uptake; also in stargazer and lethargic mice

Enhanced tonic GABA current through activation of extrasynaptic GABA-A receptors

THIP Induces SWDs in normal Wistar rats

[Graph showing time spent in seizure per 20-min period (s) and time relative to THIP injection (min)]

- aCSF (n=10)
- 70 \(\mu\)M THIP (n=5)
- 100 \(\mu\)M THIP (n=5)

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My Task: Provide basic pharmacological information to set the stage for thinking about laboratory based research to identify potential treatments for SLC6A1.

- Pharmacological therapy
- Gene therapy
Gaboxadol — a new awakening in sleep
Keith A Wafford¹ and Bjarke Ebert²

Drugs that enhance synaptic γ-aminobutyric acid (GABAergic neurotransmission are widely utilized in the clinical setting. Barbiturates and benzodiazepine receptor agonists, for example, both potentiate an inhibitory chloride conductance through GABA-gated channels, and thereby achieve their sedative-hypnotic effects. The primary locus of action of these agents, and indeed most neuroactive drugs, is the postsynaptic junction. By contrast, gaboxadol, a selective extrasynaptic GABA receptor agonist and late-stage investigational treatment for insomnia, acts on a unique δ-containing GABAΔ₆ receptor subtype found exclusively outside of the synapse. Although the mechanistic details of extrasynaptic neurotransmission remain to be fully established, it is now clear that these receptors demonstrate unique pharmacological, biophysical and electrophysiological properties. Importantly, the δ-containing GABAΔ₆ receptor subtype activated by gaboxadol is highly expressed in the thalamus, where it might behave as a ‘gain control’ (independently controlling the strength of signals) in the corticothalamic pathways that govern sleep-related neuronal oscillations. This unique mechanism has contributed to our increased understanding of sleep mechanisms, and targeting of this system offers potential advantages over existing insomnia treatments.

Addresses
¹ Merck, Sharp & Dohme, Neurosciences Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2RR, UK
² H Lundbeck A/S, Valby, Denmark

Corresponding author: Wafford, Keith A (keith.wafford@merck.com)

Current Opinion in Pharmacology 2006, 6:30-36
This review comes from a themed issue on Neurosciences
Edited by Alan Foster and John Kemp
Available online 20th December 2005
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DOI 10.1016/j.coph.2005.10.004

Introduction
The major mechanism for neuronal inhibition in the adult mammalian central nervous system utilizes γ-aminobutyric acid (GABA) receptors to reduce and control cell excitability. Playing such an important role has made GABAΔ₆ receptors prime targets for therapeutic agents that cause sedation and hypnosis via enhancement of this

are a heterogeneous family of ligand-gated ion channels selectively permeable to chloride, and can be subdivided by their molecular composition. GABAΔ₆ receptors are composed of five subunits, with current evidence supporting the combination of two α, two β and one additional subunit, either γ, δ or ε. The α, β and γ subunits can be subdivided into α₁-6, β₁-3 and γ₁-3. The >20 receptor subtypes that have now been identified are thought to have distinct functions, as suggested by their unique pharmacological and biophysical properties and their cell- and region-specific distribution patterns [1,2].

Benzodiazepines
Benzodiazepines are the most widely used group of hypnotics and mediate their effects through allosteric binding and potentiation of GABAΔ₆ receptor-mediated chloride conductance. The majority of clinically used benzodiazepines bind with high affinity only to those receptors containing a γ₂ subunit, the binding pocket of which is located between the α and γ subunit [3]. The majority of prescribed benzodiazepines behave as high-efficacy positive allosteric modulators with very little selectivity for α₁, α₂, α₃ or α₅-containing subtypes. Several benzodiazepine site ligands such as zolpidem and zaleplon demonstrate some binding selectivity for the α₁-containing subtype, now thought to be responsible for mediating the sedative component of benzodiazepine action [4,5]. These are now highly prescribed for insomnia, as are older non-selective benzodiazepines such as lorazepam and diazepam [6]. Despite being effective sedatives, benzodiazepine ligands produce tolerance if taken for longer than a few days and are addictive if taken over a long period of time, producing withdrawal symptoms on cessation [7].

The ability to generate mice with targeted deletions in receptor subunits (knockout) or with mutations that affect function (knock-in) has facilitated our understanding of the role of GABA receptor subtypes in sedative and hypnotic action. For example, the identification of a histidine residue in α₁, α₂, α₃ and α₅ subunits that is crucial for benzodiazepine binding has led to the generation of mice with individual subtypes insensitive to diazepam [4,5,8]. α₁-containing receptors might contribute to sedation, [4,5] in line with the marked sedative properties of α₁-selective ligands. α₂ and α₃ subunits have been linked to anxiolytic effects [8,9], whereas α₅-containing receptors might play a role in cognitive liability [10].

Limited or variable efficacy
Psychiatric side effects at supratherapeutic doses in abuse liability in drug abusers
Single Channel Currents in Excised Outside-Out Patches from Recombinant GABA_\(\alpha\) Receptor Transfected Cells

\[\alpha_1\beta_1\gamma_2L\]

50 \(\mu\)M GABA

+10 nM

+1 \(\mu\)M

\[\alpha_1\beta_1\]

GABA (1 \(\mu\)M) + 5\(\alpha_2\),3\(\alpha_2\)-P (.1 \(\mu\)M) + Pentobarb (50 \(\mu\)M)
Cl− – A−

Hyperpolarize

$V_m$
32

Extrasynaptic

Synaptic

+10 nM THDOC

>100 μM SR-95531

+100 nM THDOC

>100 μM SR-95531

10 pA

10 s

20 pA

10 s

GABA

Benzodiazepine

GABA<sub>A</sub> Receptor Cl⁻ Channel Complex

α

β

γ

δ

Glu

SSA

GAD

GABA

0.1 μM

1 μM

2 μM

Control

20 ms