

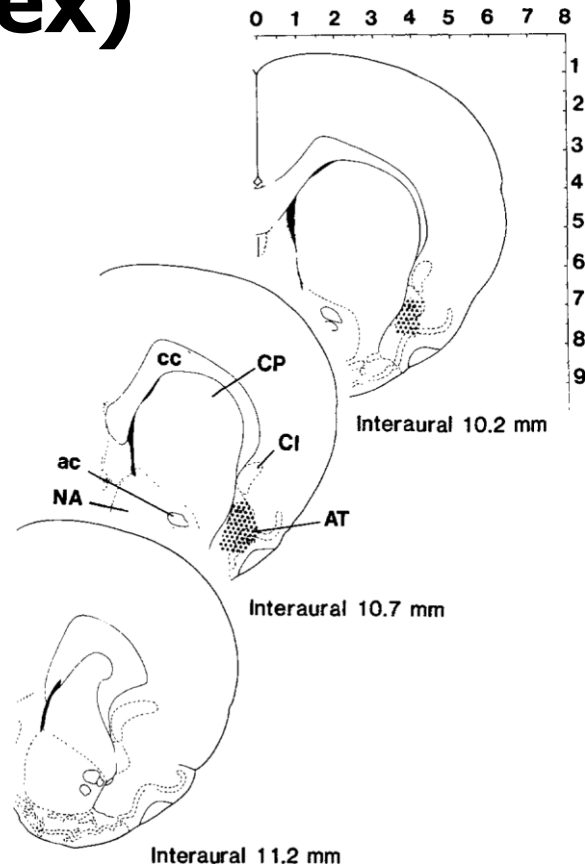


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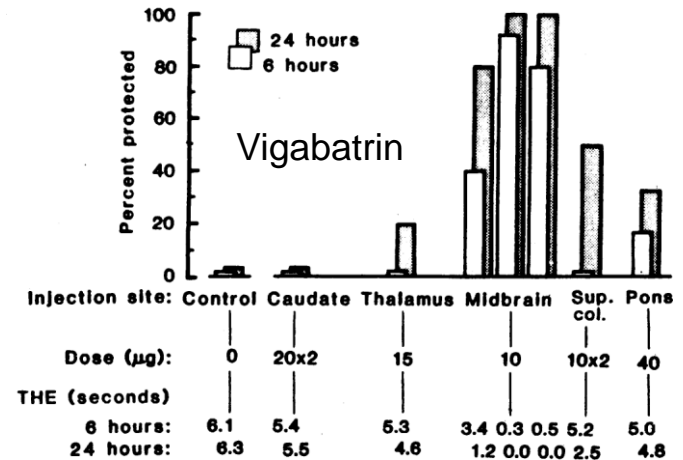
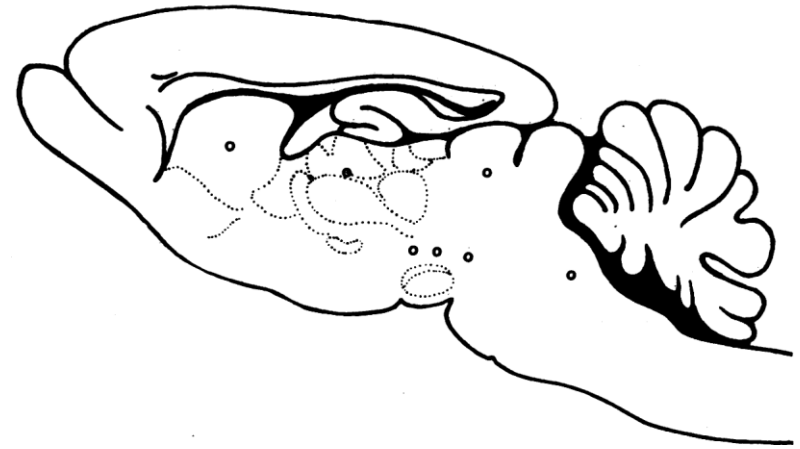
Karen Gale, PhD (1948-2014)

- 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)



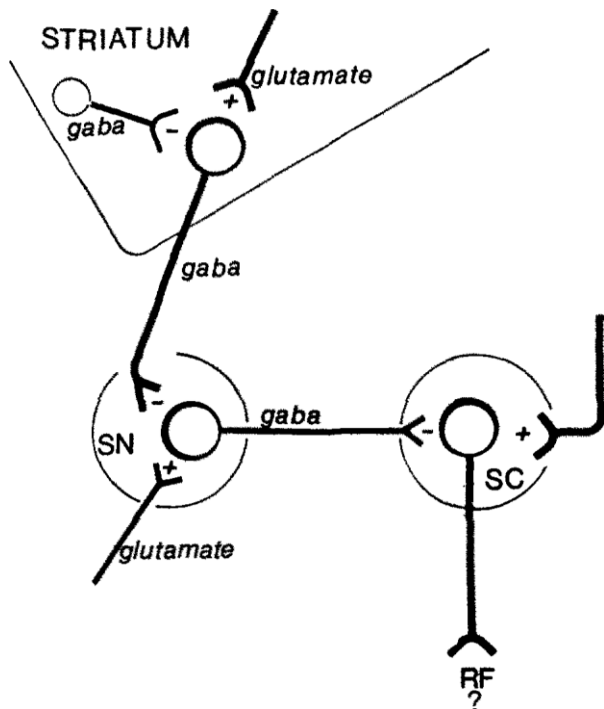
Substantia Nigra (midbrain)



Maggio R, Gale K. Seizures evoked from area tempestas are subject to control by GABA and glutamate receptors in substantia nigra. *Exp Neurol.* 1989 Aug;105(2):184-8.

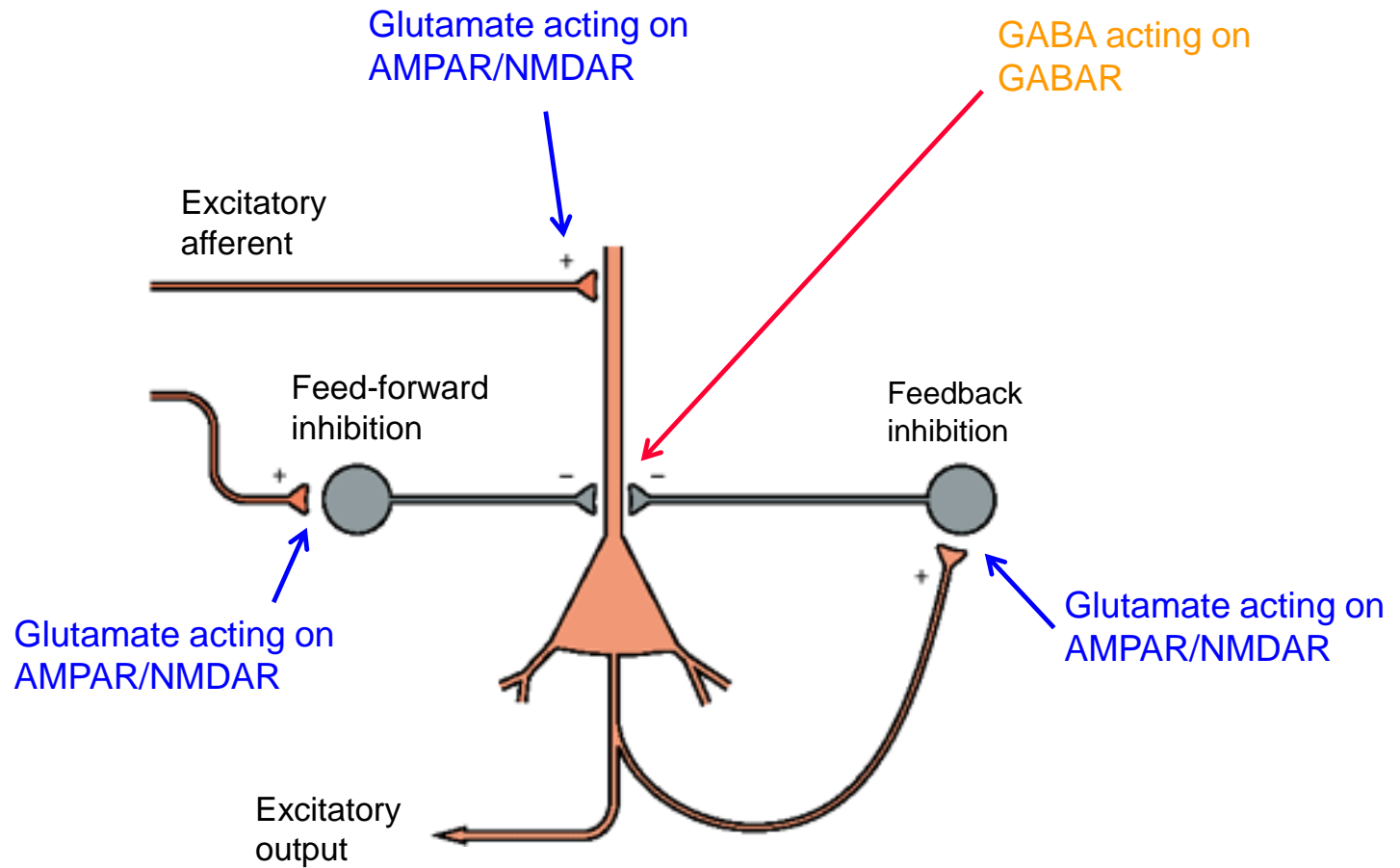
Iadarola MJ, Gale K. Substantia nigra: site of anticonvulsant activity mediated by gamma-aminobutyric acid. *Science.* 1982 Dec 17;218(4578):1237-40.

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



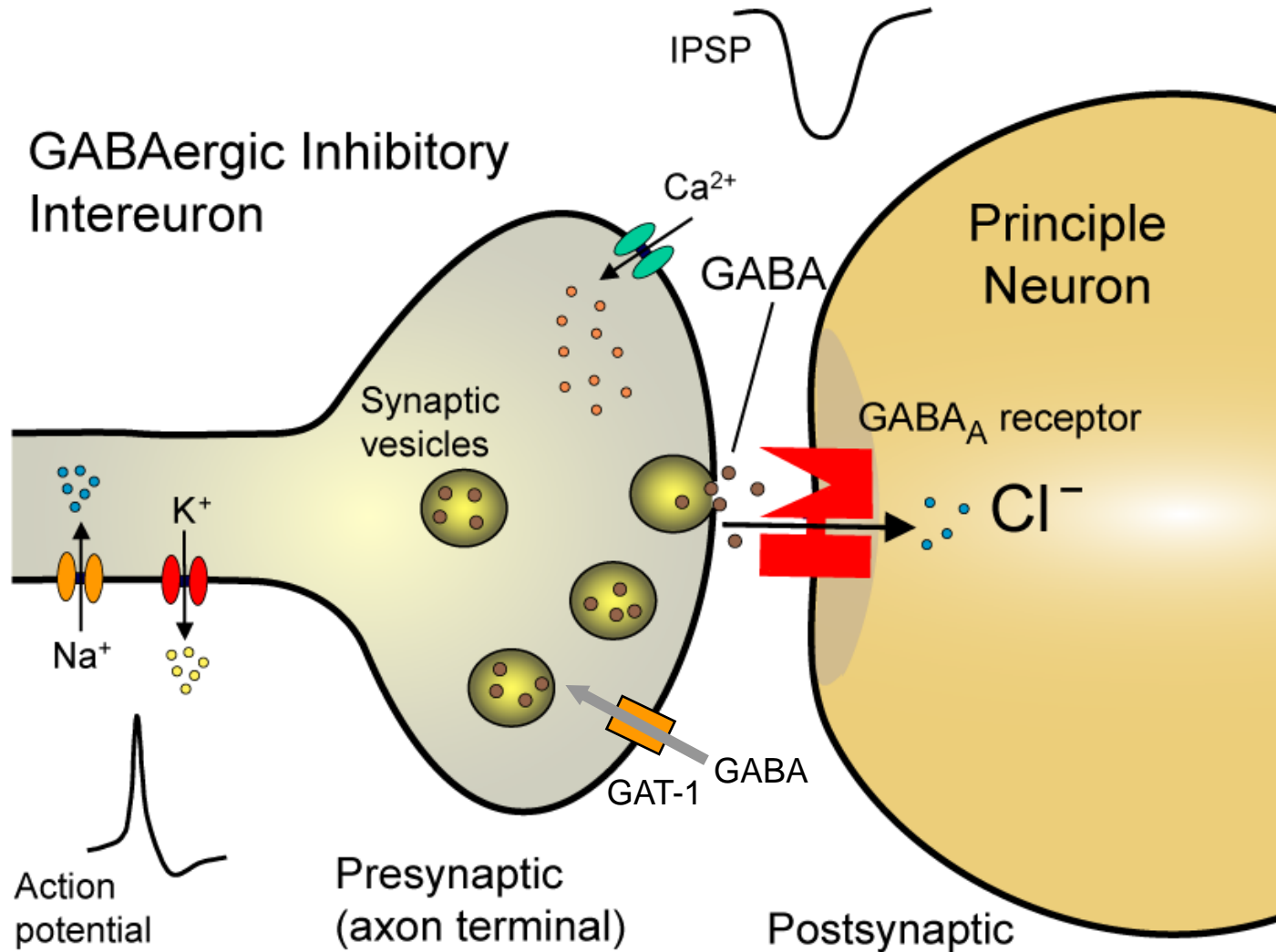
- Specific convulsant sites: "area tempestas" (deep prepiriform cortex); inferior colliculus.
- Specific anticonvulsant sites: superior colliculus

Basic Cortical Circuit

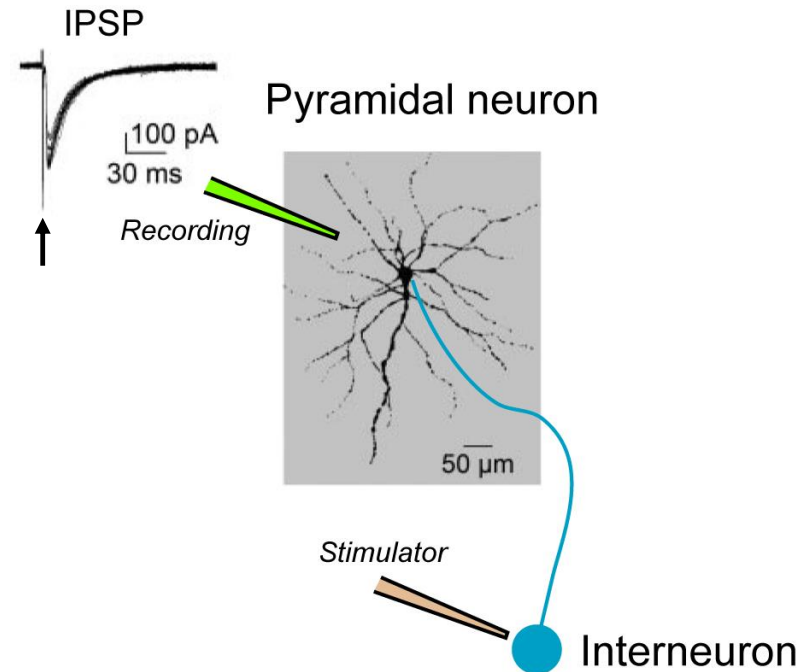
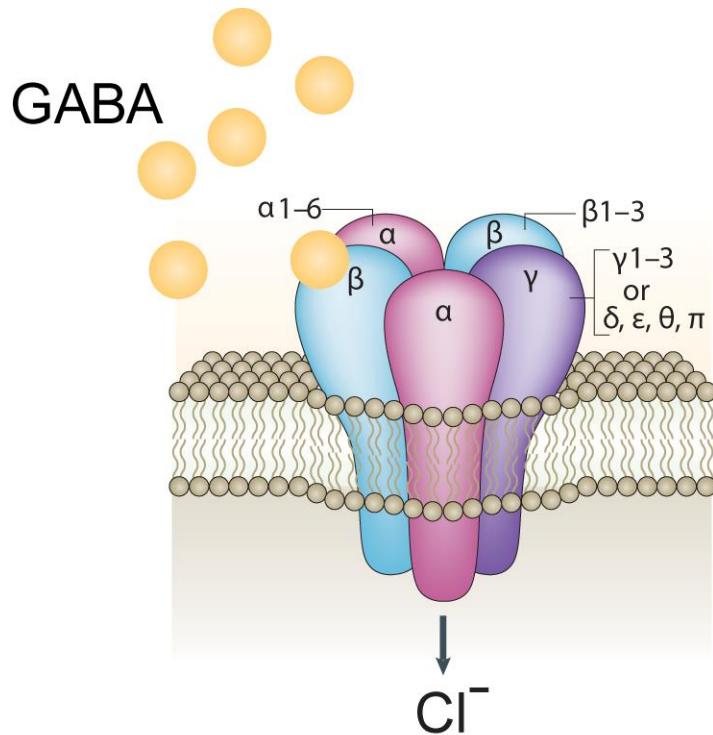


GABAR = GABA receptor
AMPA = AMPA receptor
NMDAR = NMDA receptor

GABAergic Synapse

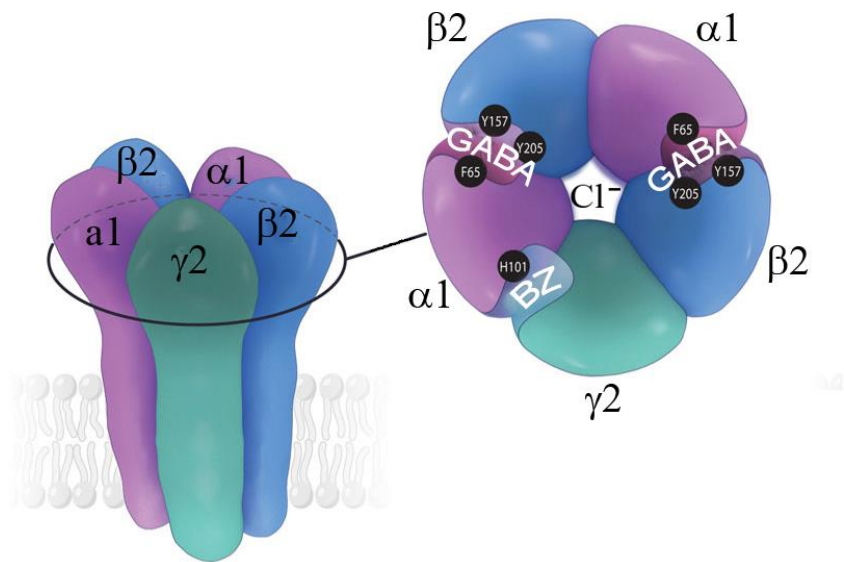


Subunit Architecture and Physiological Role of GABA_A Receptors



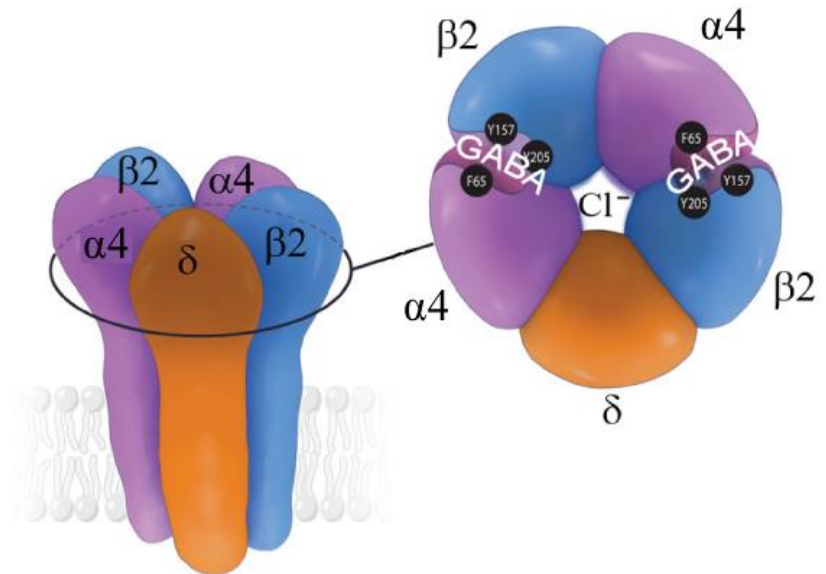
Pharmacology of Synaptic and Extrasynaptic GABA_A Receptors

Synaptic



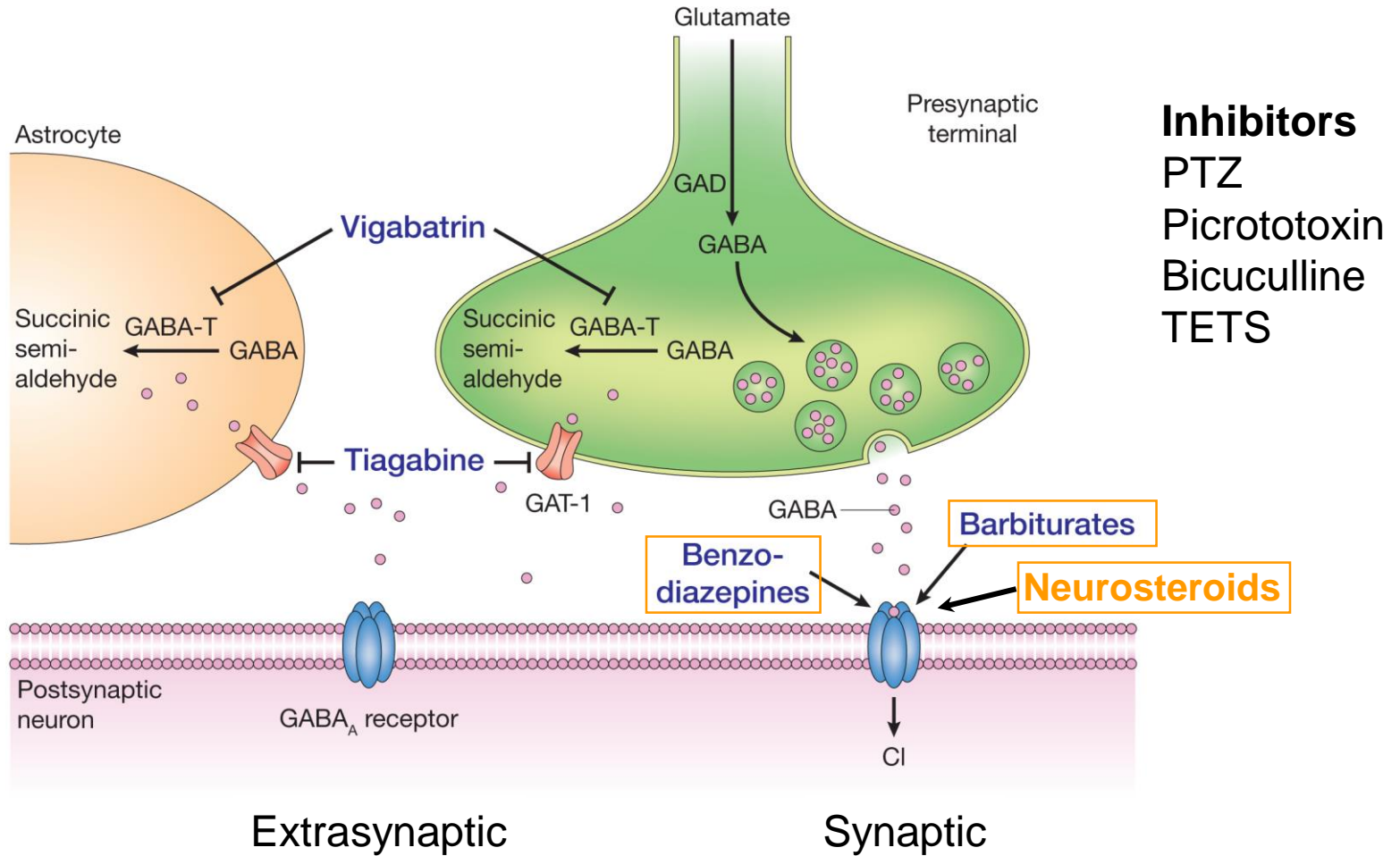
Benzodiazepines, zolpidem active
Neurosteroids active

Extrasynaptic



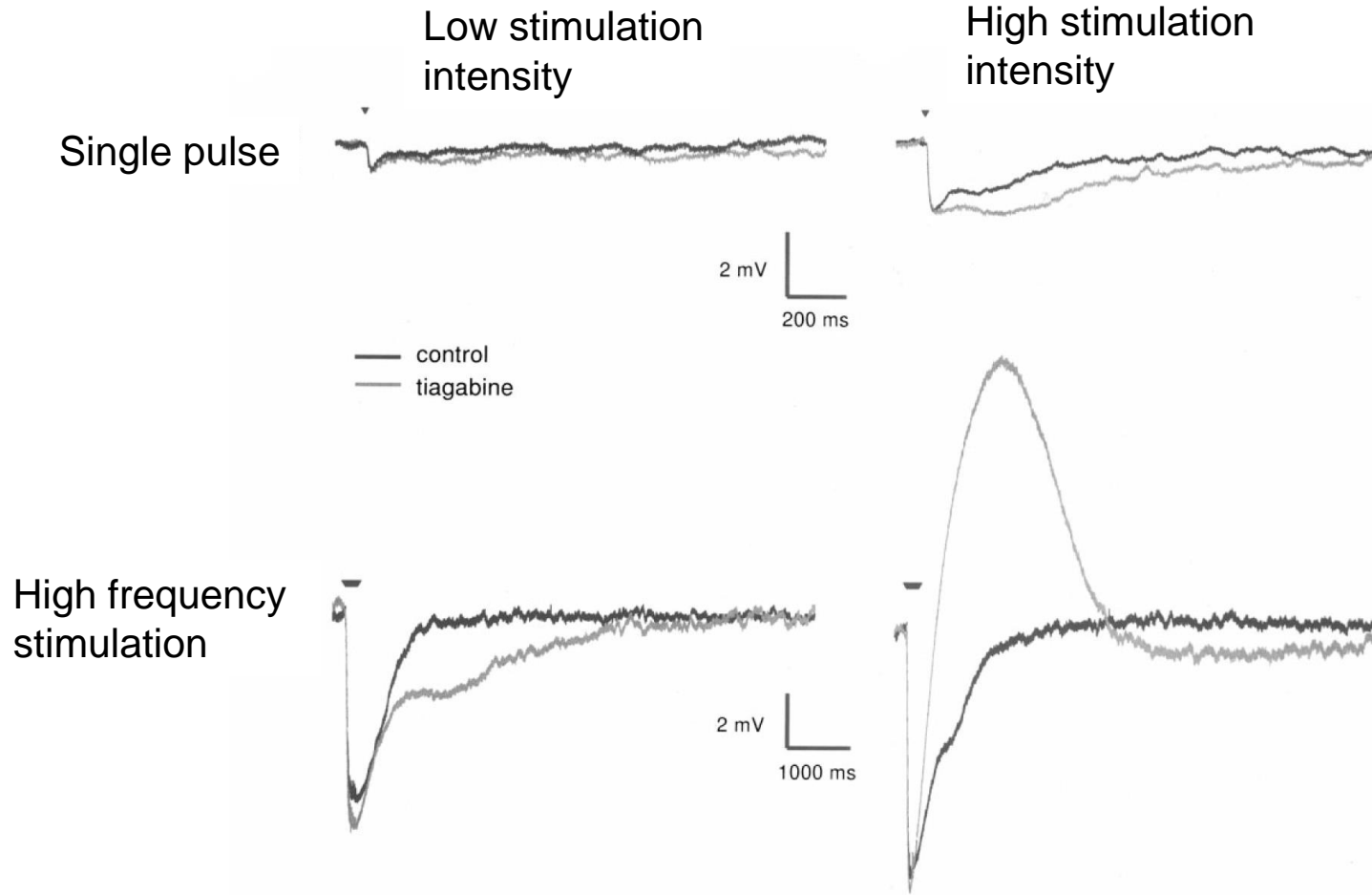
Benzodiazepines, zolpidem inactive
Neurosteroids active

Inhibitory Synapse



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 Spiller^{1,2}, D Wiles¹, JL Russell¹ and MJ Casavant^{1,2}

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 2008, 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.^{1–9} 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.^{9,10} An atypical

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

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

SZ: seizures.

CASE REPORT

Tiagabine-induced absence status in idiopathic generalized epilepsy

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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 epilepsy¹. 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 encountered². The incidence of absence status in patients with absence epilepsy is estimated to be between 2 and 10%³.

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 synapses⁴. It increases the extracellular concentration of GABA after oral administration^{5,6}. In clinical trials TGB had a dose-related anticonvulsant effect in patients with focal epilepsies^{7–11}. However, in animal models the frequency of interictal spike-

wave discharges increased in rats when treated with TGB^{12–14}.

In addition, TGB was also reported to induce non-convulsive status epilepticus in several patients with partial epilepsy^{15,16} and in one patient with juvenile myoclonic epilepsy⁶. 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

32-year-old patient with absence epilepsy and primary generalized TC seizures developed first absences status epilepticus when treated with tiagabine

mGAT1 KO Mouse Phenocopies Some Effects of Tiagabine

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

3234 • The Journal of Neuroscience, March 23, 2005 • 25(12):3234–3245

Neurobiology of Disease

GABA Transporter Deficiency Causes Tremor, Ataxia, Nervousness, and Increased GABA-Induced Tonic Conductance in Cerebellum

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GABA transporter subtype 1 (GAT1) knock-out (KO) mice display normal reproduction and life span but have reduced body weight (female, ~10%; male, ~20%) and higher body temperature fluctuations in the 0.2–1.5/h frequency range. Mouse GAT1 (mGAT1) KO mice exhibit motor disorders, including gait abnormality, constant 25–32 Hz tremor, which is aggravated by flunitrazepam, reduced rotarod performance, and reduced locomotor activity in the home cage. Open-field tests show delayed exploratory activity, reduced rearing, and reduced visits to the central area, with no change in the total distance traveled. The mGAT1 KO mice display no difference in acoustic startle response but exhibit a deficiency in prepulse inhibition. These open-field and prepulse inhibition results suggest that the mGAT1 KO mice display mild anxiety or nervousness. The compromised GABA uptake in mGAT1 KO mice results in an increased GABA_A receptor-mediated tonic conductance in both cerebellar granule and Purkinje cells. The reduced rate of GABA clearance from the synaptic cleft is probably responsible for the slower decay of spontaneous IPSCs in cerebellar granule cells. There is little or no compensatory change in other proteins or structures related to GABA transmission in the mGAT1 KO mice, including GAT1-independent GABA uptake, number of GABAergic interneurons, and GABA_A⁺ vesicular GABA transporter-, GAD65-, and GAT3-immunoreactive structures in cerebellum or hippocampus. Therefore, the excessive extracellular GABA present in mGAT1 KO mice results in behaviors that partially phenocopy the clinical side effects of tiagabine, suggesting that these side effects are inherent to a therapeutic strategy that targets the widely expressed GAT1 transporter system.

Key words: tiagabine; epilepsy; flunitrazepam; cerebellum; inhibition; tremor

Introduction

GABA is the principal inhibitory neurotransmitter in the mammalian brain, where it activates GABA_A, GABA_B, and GABA_C receptors. GABA released from presynaptic terminals is removed from the vicinity of the synaptic cleft by GABA transporters, and this action is believed to be a key event in terminating synaptic currents. GABA transporters are also involved in maintaining a low extracellular GABA concentration throughout the brain, preventing excessive tonic activation of synaptic and extrasynaptic receptors. GABA transporters may also play a role in replenishing the supply of presynaptic transmitter. Furthermore, GABA trans-

porters may reverse, under both normal and pathological circumstances, to release GABA (Richerson and Wu, 2003, 2004).

Of the three GABA transporters identified in the CNS, GABA transporter subtype 1 (GAT1) is highly expressed in the olfactory bulb, neocortex, cerebellum, superior colliculus, and substantia nigra, where it is predominantly found in axons, presynaptic terminals, and glial cells. GAT2 is weakly expressed throughout the brain, primarily in arachnoid and ependymal cells. GAT3 expression is densest in the olfactory bulb, midbrain regions, and deep cerebellar nuclei, where it is found predominantly on glial cells (Radian et al., 1990; Ikegaki et al., 1994; Itouji et al., 1996; Yan et al., 1997; Engel and Wu, 1998; Barakat and Bordey, 2002; Chiu et al., 2002).

The GAT1 inhibitor tiagabine is a clinically useful antiepileptic drug with few cognitive side effects (Aldenkamp et al., 2003), but it also causes tremor (its major side effect), ataxia, dizziness, asthenia, somnolence (sedation), and nonspecific nervousness (Adkins and Noble, 1998; Pellock, 2001; Schachter, 2001). It is important to know whether these side effects arise directly from increased extracellular concentration of GABA in the CNS or, instead, from actions on unintended targets. For instance, GAT1

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

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Anticonvulsant Activity of Intravenous Muscimol in Rats

Neuropharmacology, Vol. 18, pp. 885 to 889
Pergamon Press Ltd 1979. Printed in Great Britain

ANTICONVULSANT ACTIVITY OF MUSCIMOL AGAINST SEIZURES INDUCED BY IMPAIRMENT OF GABA-MEDIATED NEUROTRANSMISSION

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(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 metrazole 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 > phenytoin. Muscimol had no effect on strychnine-induced convulsions. It is concluded that muscimol penetrates rat brain and specifically antagonizes seizures caused by GABA receptor blockade or depletion of brain GABA.

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 Tower, 1976; DeFeudis, 1977). Experimental evidence implicates a hypofunctional central GABA system in certain neurologic 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 (Wheal and Kerkut, 1976) and at bicuculline-sensitive postsynaptic receptors in the mammal (Krogsgaard-Larsen, Johnston, Curtis, Game and McCulloch, 1975; Enna, Collins and Snyder, 1977). A delay in the onset of isoniazid-induced seizures after muscimol treatment has been reported (Naik, Guidotti 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.

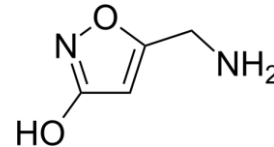
Drugs

Seizures were produced by systemic administration of the following convulsants: picrotoxin (Smith Kline & French); isonicotinic acid hydrazide (Isoniazid, Sigma); bicuculline (Sigma); strychnine sulfate (Smith Kline & French); pentylenetetrazole (Metrazole, Kroll Pharmaceuticals). These compounds were tested for anticonvulsant activity: muscimol (Smith Kline & French); diazepam (Smith Kline & French); phenobarbital sodium (Smith Kline & French); diphenhydantoin sodium (Phenytoin, Sigma). Muscimol, phenobarbital, isoniazid, strychnine and picrotoxin were dissolved in water or saline. Diazepam and bicuculline were administered in dilute HCl (pH 3). Phenytoin was dissolved in water with the addition of NaOH to a final pH of 11.

Chemically-induced convulsions

Picrotoxin was injected subcutaneously into rats and the latency to onset of convulsions was measured. A mean onset time was determined in control (non-anticonvulsant treated) animals for each experimental day. A rat treated with an anticonvulsant agent was considered "protected" if it failed to convulse within two standard deviations of the mean onset time for the control group. Rats were also injected subcutaneously with isoniazid and monitored for the onset of convulsive activity. Time to onset of clonic and tonic seizure components was noted. An animal was considered "protected" if it failed to have a seizure episode within two standard deviations of the mean onset time for a control group treated only with isoniazid. Bicuculline, strychnine and pentylenetetrazole were injected intravenously. The time to onset of the components of seizure activity was recorded and the pattern of seizure development observed. Animals

Muscimol



GABA

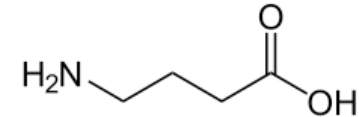


Table 4. Summary of anticonvulsant testing. Comparison of muscimol with standard antiepileptic agents

Compound	Bicuculline	Convulsant Strychnine	Pentylenetetrazole
Muscimol	1.0 (0.7–2.0)	No effect at 8 mg/kg	0.49 (0.32–1.5)
Diazepam	0.32 (0.07–0.49)	0.82 (0.4–1.4)	0.09 (0.04–0.14)
Phenobarbital	7.9 (5.1–10.7)	35.9 (22.2–63.8)	4.1 (2.2–7.4)
Phenytoin	9.3 (5.0–13.3)	136.2 (80.0–354.4)	Not tested

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.

GAT-1 Is Present in Immature Brain But Not Functionally Active



European Journal of Pharmacology 383 (1999) 259–266



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Age-dependence of the anticonvulsant effects of the GABA uptake inhibitor tiagabine in vitro

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Received 25 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 γ -aminobutyric acid (GABA) uptake inhibitor tiagabine [(*R*)-*N*-(4,4-bis(3-methyl-2-thienyl)butyl)-3-en-1-yl] nipecotic acid on low-Mg²⁺-induced epileptic discharges in brain slices from rat pups (p 5–8) and juvenile animals (p 15–20). In tissue 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 unaffected in CA1 but suppressed by 60% in the entorhinal cortex. While tiagabine increases its efficacy with age, in-situ hybridisation and PCR analysis show that mRNA 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 slice; Tiagabine; GABA (γ -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 (Moshé et al., 1983; Lothman and Bertram, 1993). Various mechanisms have been suggested to contribute to this age-dependence, including depolarising GABA_A receptor-mediated potentials (Kriegstein et al., 1987; Ben-Ari et al., 1988), immature regulation of K⁺-homeostasis in the developing brain (Hablitz and Heinemann, 1987), changes in the function and expression of NMDA receptors (Brady et al., 1991) or enhanced recurrent excitatory connections (Swann et al., 1993). These developmental characteristics may be mirrored by differences in the potency or efficacy of anticonvulsant drugs. In juvenile rats, various γ -aminobutyric acid (GABA) mimetic substances show little efficacy against

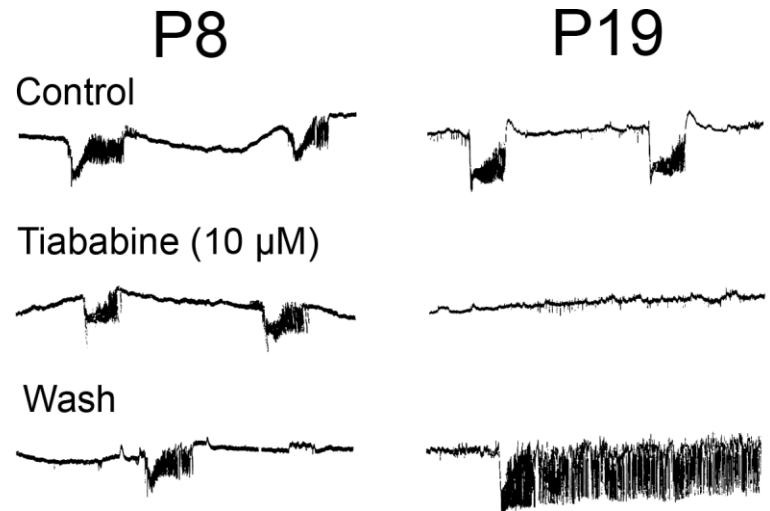
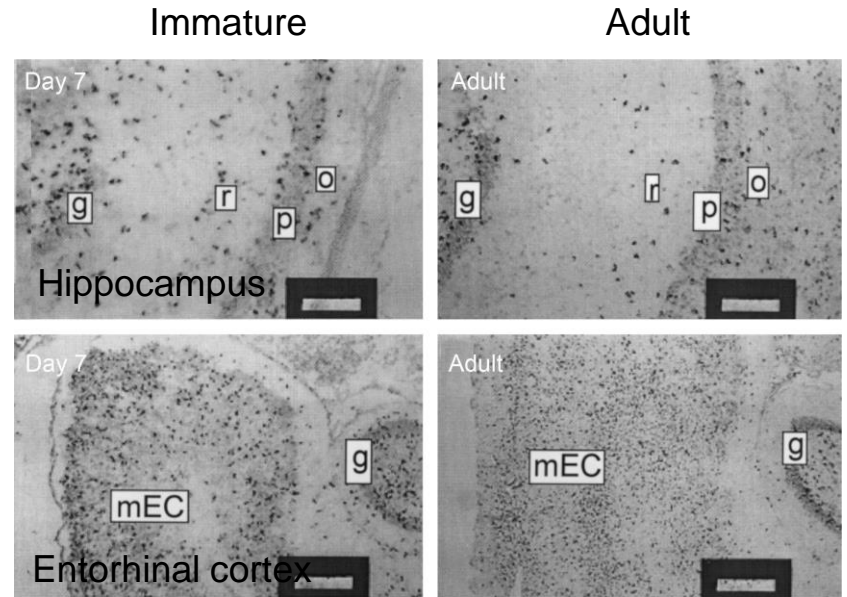
experimentally induced afterdischarges and tonic-clonic seizures (Velsick and Mares, 1995; Polásek et al., 1996). Microinfusion of GABA_A receptor agonists into the substantia nigra of rat pups exerts proconvulsant effects on flurothyl-induced seizures whereas they are anticonvulsant at low doses in adults (Garant et al., 1995). Therefore, GABAergic agents should be thoroughly tested throughout development including very immature stages.

Tiagabine [(*R*)-*N*-(4,4-bis(3-methyl-2-thienyl)butyl)-3-en-1-yl] nipecotic acid is a derivative of nipecotic acid with good anticonvulsant activity both in vitro and in vivo (Faingold et al., 1994; Suzdak, 1994; Walton et al., 1994; Smith et al., 1995; Pfeiffer et al., 1996). It is effective as add-on therapy in complex partial seizures in adults (Gram, 1994; Richens et al., 1995; Luer and Rhoney, 1998) while results from children with epilepsy are not yet sufficient to allow for a comparison with established anticonvulsants (Adkins 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 (Rekling et al., 1990; Roepstorff and Lambert, 1994). However, in a recent study on inhibitory postsynaptic currents (IPSCs) in dentate granule

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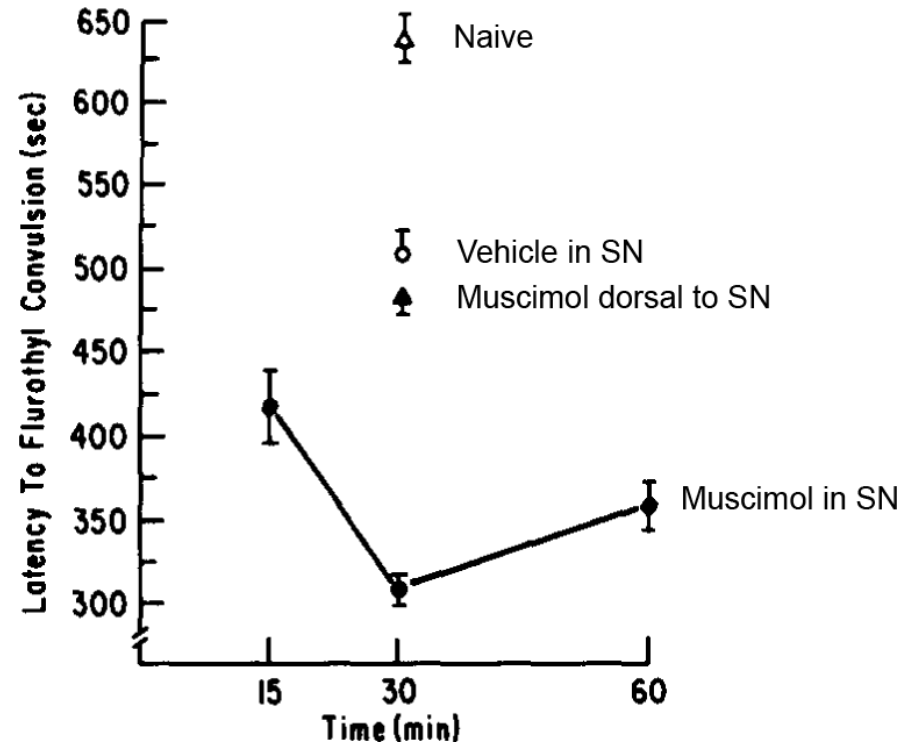
E-mail address: andreas.draguhn@charite.de (A. Draguhn)

GAT-1 In situ



Muscimol Facilitates Seizures in Immature Brain

Latency to Flurothyl Convulsion



Bilateral nigral infusion of muscimol

Age-Related Differences in the Effects of GABA_A Agonists Microinjected Into Rat Substantia Nigra: Pro- and Anticonvulsant Actions

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Summary: GABAergic transmission in the substantia nigra pars reticulata (SNR) has an important role in the control of experimental seizures. In the flurothyl seizure model, SNR microinjection of the selective GABA_A receptor agonist muscimol results in a biphasic dose-response curve in adults: Intermediate doses are anticonvulsant, but high doses have proconvulsant effects. Another GABA_A agonist, THIP (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol), 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_A receptor agonists than is the SNR of adults. We hypothesize that the age-related differences in nigral GABAergic response may be due to ontogenic changes in GABA_A-sensitive neuronal circuits in the SNR. **Key Words:** Muscimol—THIP—Flurothyl—Seizures—Epilepsy— γ -Aminobutyric 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 neuroscience research is to identify the neuronal circuitry and synaptic pharmacology underlying this ontogenic phenomenon. Such understanding will help direct the development of more efficacious and selective age-specific therapies than those currently available.

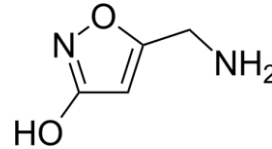
The substantia nigra pars reticulata (SNR) has been identified as a critical site in rat brain for the anticonvulsant action of GABAergic drugs (2). Since it was shown that GABAergic transmission in SNR can attenuate seizures (3,4), a series of investigations (5,6) led to the hypothesis that experimental 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.

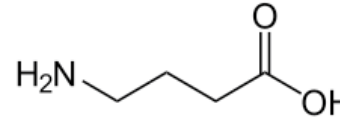
Our laboratory has studied developmental differences in the anticonvulsant pharmacology of GABA transmission in rat SNR. We measured the susceptibility of rats to seizures induced by flurothyl (hexafluoroethyl ether), a volatile inhaled convulsant well suited to developmental studies (9). We commonly 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 flurothyl seizures of some GABAergic agents to be similar regardless of the age of the rats examined. Doses of the presynaptic GABA agonist γ -vinyl-GABA (GVG) that increase GABA in SNR result in the suppression of flurothyl seizures in both adult (10) and 16-day-old rats (11). Conversely, microinjections into SNR of bicuculline, a competitive GABA_A receptor antagonist (12) significantly facilitate flurothyl seizures in both adults (13) and pups

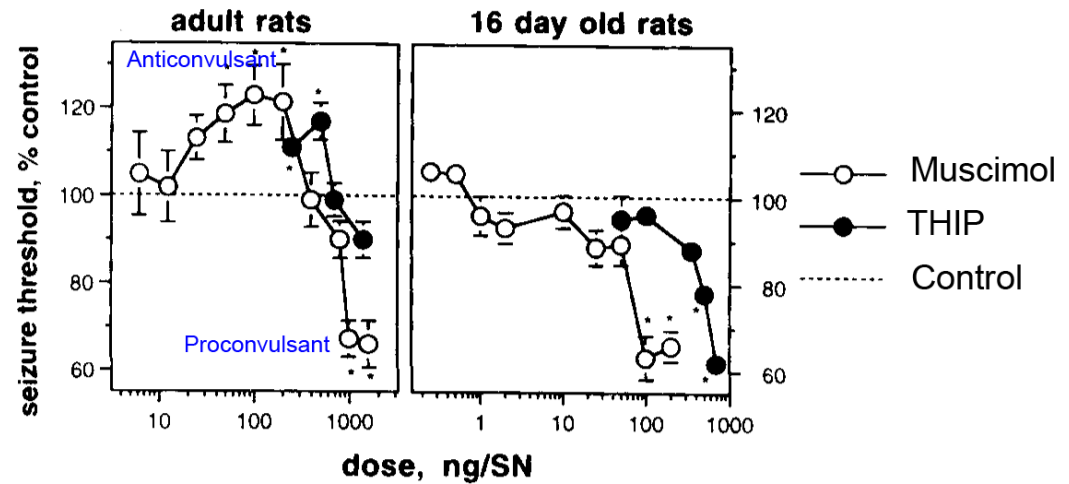
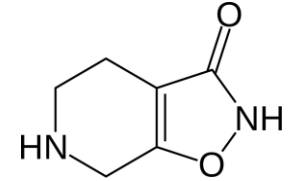
Muscimol



GABA



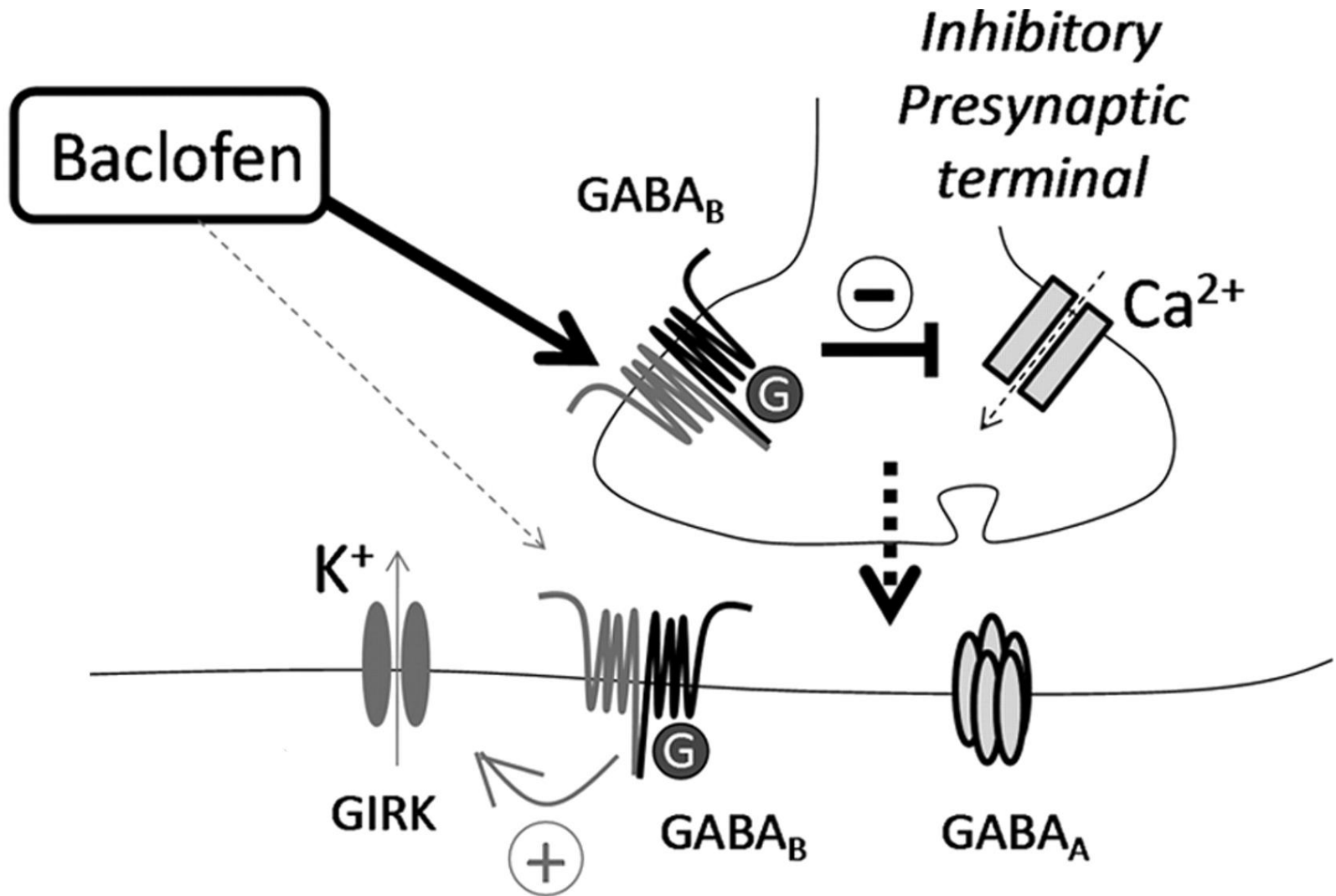
Gaboxadol (THIP)



Received August 27, 1994; revision accepted April 13, 1995. Address correspondence and reprint requests to Dr. S. L. Moshé at Rose F. Kennedy Center, Room 316, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 U.S.A.

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GABA_B Receptors



CHARACTERIZATION OF GABAergic SEIZURE REGULATION IN THE MIDLINE THALAMUS

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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 agonists, 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_B 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 hyperactivity 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 origination 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.

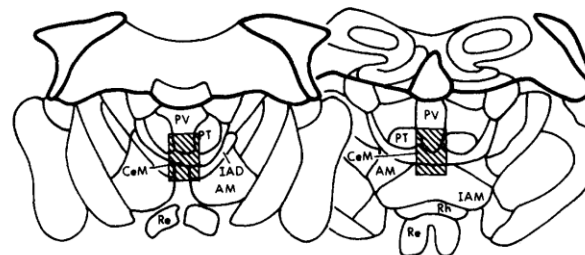
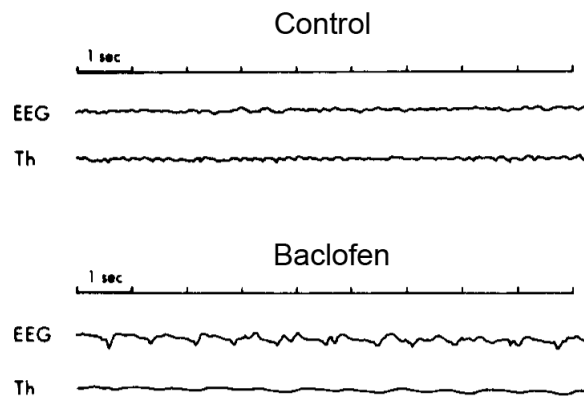
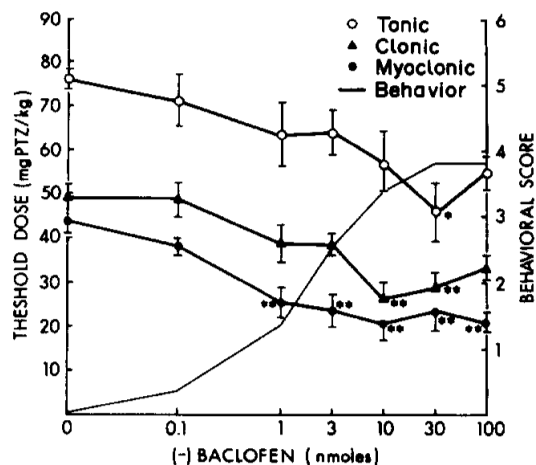


Fig. 1. Diagram showing the target zone in the central medial nucleus in the thalamus. For inclusion in the study, the entire blue injection site had to be confined to this zone. Abbreviations are as follows: AM—anteromedial nucleus; CeM—central medial nucleus; IAD—interanterodorsal nucleus; IAM—interanteromedial nucleus; PT—paratenial nucleus; PV—paraventricular nucleus; Re—reunions; Rh—rhomboid nucleus.

Injection of Baclofen into Midline Thalamus



Proepileptic Activity of Baclofen is Due to Disinhibition

GABA_B autoreceptor-mediated cell type-specific reduction of inhibition in epileptic mice

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Contributed by Nancy J. Kopell, July 20, 2013 (sent for review February 2, 2013)

GABA_B receptors (GABA_BRs) mediate slow inhibitory effects on neuronal excitability and synaptic transmission in the brain. However, the GABA_BR agonist baclofen can also promote excitability and seizure generation in human patients and animal models. Here we show that baclofen has concentration-dependent effects on the hippocampal network in a mouse model of mesial temporal lobe epilepsy. Application of baclofen at a high dose (10 mg/kg i.p.) reduced the power of γ oscillations and the frequency of pathological discharges in the Cornu Ammonis area 3 (CA3) area of freely moving epileptic mice. Unexpectedly, at a lower dose (1 mg/kg), baclofen markedly increased γ activity accompanied by a higher incidence of pathological discharges. Intracellular recordings from CA3 pyramidal cells in vitro further revealed that, although at a high concentration (10 μ M), baclofen invariably resulted in hyperpolarization, at low concentrations (0.5 μ M), the drug had divergent effects, producing depolarization and an increase in firing frequency in epileptic but not control mice. These excitatory effects were mediated by the selective muting of inhibitory cholecystinin basket cells (CCK⁺ BCs), through enhanced inhibition of GABA release via presynaptic GABA_BRs. We conclude that cell type-specific up-regulation of GABA_BR-mediated autoinhibition in CCK⁺ BCs promotes aberrant high frequency oscillations and hyperexcitability in hippocampal networks of chronic epileptic mice.

presynaptic inhibition | mTLE model | patch clamp

Neuronal activity in the hippocampus shows oscillations in behavior-relevant frequency ranges including γ frequencies (30–80 Hz) (1). γ activity is prominent in the aroused brain and has been implicated in higher-level brain functions, such as sensory binding, perception (2), and storage and recall of information (3, 4). At the same time, γ frequency oscillations are also prevalent in epileptic patients and are most often observed at seizure onset during in depth EEG recordings (5). The GABAergic system plays a pivotal role in the generation of γ oscillations (6–8). However, it remains to be resolved how distinct GABAergic receptor subtypes, in particular GABA_A receptors (GABA_ARs), contribute to the generation and modulation of pathological network oscillatory activity.

GABA_BRs mediate slow inhibitory effects and control synaptic transmission and the excitability of neurons in cortical networks. GABA_BRs are expressed both postsynaptically in somatodendritic compartments and presynaptically in axon terminals, in excitatory principal cell and inhibitory interneurons (9–11). The effects of GABA_BR activation on the network are dominated by inhibition leading to an overall dampened population activity. However, if GABAergic interneurons are affected dominantly, as observed for example, during high-frequency stimulation, GABA_BR activation can produce disinhibition in principal cells (12, 13). Accordingly, the role of GABA_BRs in epilepsy and seizure generation remains ambiguous. GABA_BRs are expected to have an overall antiepileptic effect, and indeed, the receptor KO

animals show an epileptic phenotype (14). However, there is also evidence that the receptor agonist baclofen can induce seizures in patients after intrathecal application (15, 16). The picture is further complicated by the fact that GABA_BR expression can be altered in both epileptic patients, e.g., in mesial temporal lobe epilepsy (mTLE) (17), and animal models (18). Thus, cell type-specific alterations in GABA_BR expression may change network excitability during the progression of mTLE.

Using a chronic kainate (KA) model of mTLE, which reproduces major electrophysiological and histopathological characteristics of human mTLE (19, 20), we studied the role of GABA_BRs in altered hippocampal network activity. Our results suggest that enhanced and persistent GABA_BR activation in epileptic mice suppresses the inhibitory output from hippocampal interneurons, in particular cholecystinin (CCK)-expressing basket cells (BCs) onto pyramidal cells (PCs). This reduction in the inhibitory output of interneurons, in turn, leads to disinhibition in hippocampal networks, enhances γ activity, and promotes the transition to pathological hyperexcitability.

Results

Dose-Dependent Effects of GABA_BR Activation on Hippocampal Network Activity in a Mouse Model of mTLE in Vivo. We first analyzed the effects of GABA_BR activation on network activity in vivo in chronic epileptic mice. EEG recordings were carried out

Significance

Metabotropic 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 proepileptic 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 synaptic output of a specific set of inhibitory neurons. This disinhibitory effect promotes high-frequency oscillations and the emergence of pathological discharges in the epileptic hippocampal network.

Author contributions: T.G. designed research; T.D., N.M., C.B., S.G., U.H., A.W., and T.G. performed research; U.H. and C.A.H. contributed new reagents/analytic tools; T.D., N.M., C.B., S.G., J.C.M., I.V., N.J.K., and T.G. analyzed data; and T.D., C.B., J.C.M., I.V., N.J.K., and T.G. wrote the paper.

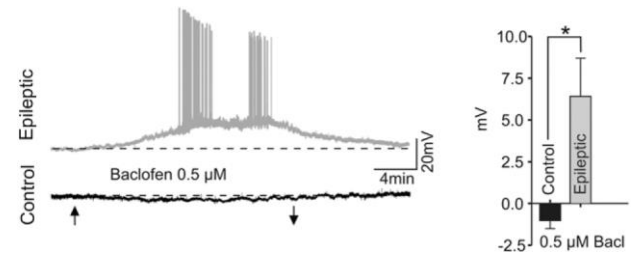
The authors declare no conflict of interest.

¹T.D. and N.M. contributed equally to this work.

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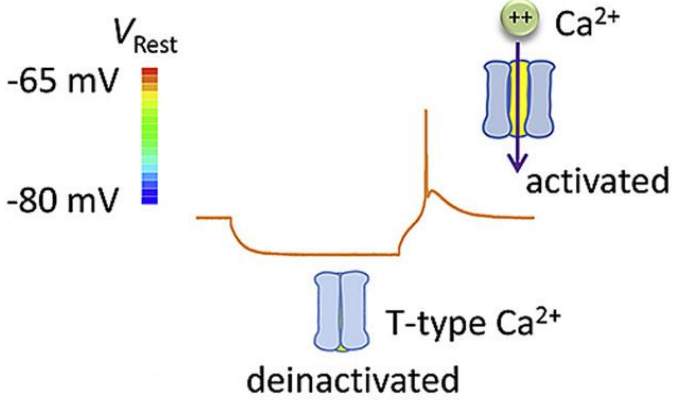
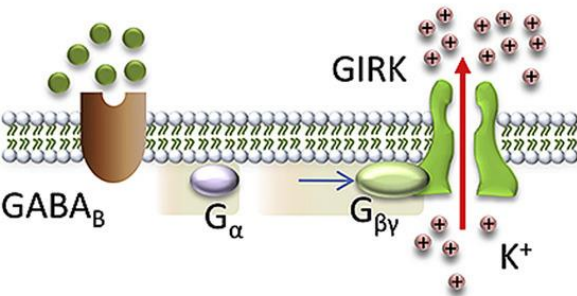
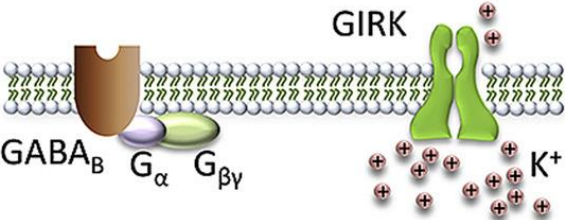
This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1313505110/-DCSupplemental.

Chronic kainate model mTLE



- 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 GABA_B receptor activation
- Enhanced activation of presynaptic GABA_B receptors leads decreased GABA release and consequent disinhibition and hyperexcitability of pyramidal cells.

GABA_B Receptors



The End

Enhanced tonic GABA_A inhibition in typical absence epilepsy

David W Cope¹, Giuseppe Di Giovanni^{1,2}, Sarah J Fyson¹, Gergely Orbán^{1,2}, Adam C Errington¹, Magor L Lőrincz¹, Timothy M Gould¹, David A Carter¹ & Vincenzo Crunelli¹

The cellular mechanisms underlying typical absence seizures, which characterize various idiopathic generalized epilepsies, are not fully understood, but impaired γ -aminobutyric acid (GABA)-ergic inhibition remains an attractive hypothesis. In contrast, we show here that extrasynaptic GABA_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 seizure genesis. Extrasynaptic GABA_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_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 epileptogenic importance and highlight potential therapeutic targets for the treatment of absence epilepsy.

Typical absence seizures characterize numerous idiopathic generalized epilepsies and appear in the electroencephalogram (EEG) as bilaterally synchronous spike-and-wave discharges (SWDs) accompanied by behavioral arrest^{1,2}. Whereas absence seizures are known to arise in thalamo-cortical networks^{3–5}, the underlying cellular mechanisms are not fully understood. Impaired GABAergic inhibition remains an attractive hypothesis^{3,6}, and GABA_A receptor (GABA_AR) subunit mutations have been identified in human cohorts with typical absence seizures, albeit as part of a complex phenotype^{7–9}. However, although some of these mutations compromise GABA_AR function in heterologous expression systems¹⁰, only modest changes in GABA_AR inhibition have so far been identified in the thalamo-cortical network of rodents with spontaneous SWDs^{11–13}. Furthermore, systemic or intrathalamic administration of agents that promote GABAergic inhibition, including the antiepileptic drugs vigabatrin and tiagabine, initiate or exacerbate seizures in humans and rodents^{14–18}. Thus, augmented rather than impaired GABA_AR inhibition may be a feature of absence seizures.

Activation of GABA_ARs generates two types of inhibition: the transient activation of synaptic GABA_ARs (sGABA_ARs) elicits inhibitory postsynaptic currents (IPSCs), or 'phasic' inhibition; and the activation of peri- or extrasynaptic GABA_ARs (eGABA_ARs) by ambient GABA causes a persistently active, or tonic, current^{19,20}. Because in thalamocortical neurons, major players in thalamo-cortical networks during SWDs, >90% of GABA_AR inhibition is tonic^{21–24}, we have examined the prospect of aberrant tonic inhibition in experimental absence seizures. Our data indicate that enhanced tonic GABA_A inhibition is a common feature of diverse genetic and

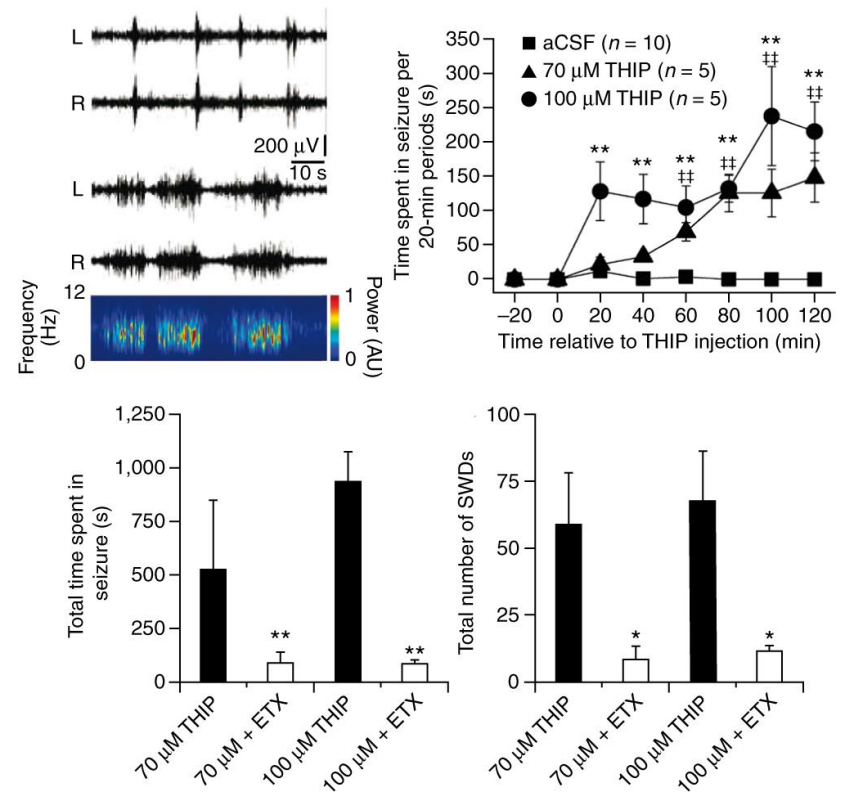
pharmacological models of typical absence epilepsy and may be a requirement for the appearance of absence seizures.

RESULTS

Enhanced tonic GABA_A current in genetic models of absence

Genetic absence epilepsy rats from Strasbourg (GAERS) are a well established polygenic model of absence epilepsy that show bilateral spontaneous SWDs and accompanying behavioral arrest from approximately postnatal day 30 (P30)¹⁶. In thalamocortical neurons, tonic GABA_A currents are generated by extrasynaptic receptors containing the δ subunit^{21–23}, and, in rats, δ subunit expression is apparent only from approximately P12 (ref. 25). Therefore, we measured tonic GABA_A current amplitude from thalamocortical neurons in slices of the somatosensory ventrobasal thalamus of GAERS from P14 onward and compared it to non-epileptic control (NEC) rats of the same age. We observed no significant difference in tonic current amplitude at P14–P16 ($P > 0.05$ for each day) (Fig. 1a,b). At P17, 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 capacitance (Supplementary Results and Supplementary Fig. 1a). 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 GAERS¹². Notably, there was a significantly ($P < 0.05$) smaller sIPSC peak amplitude, frequency, charge transfer and total current in GAERS at P18, but these changes were not maintained at later ages (Supplementary Table 1). These results

THIP Induces SWDs in normal Wistar rate



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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 **ex**trasynaptic GABA-A receptors

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



ELSEVIER

Gaboxadol — a new awakening in sleep

Keith A Wafford¹ and Bjarke Ebert²

Drugs that enhance synaptic γ -aminobutyric acid (GABA)ergic 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 GABA_A 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 GABA_A 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-relevant 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.

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Introduction

The major mechanism for neuronal inhibition in the adult mammalian central nervous system utilizes γ -aminobutyric acid (GABA)_A receptors to reduce and control cell excitability. Playing such an important role has made GABA_A 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 sub-classified by their molecular composition. GABA_A 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 α 1–6, β 1–3 and γ 1–3. 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].

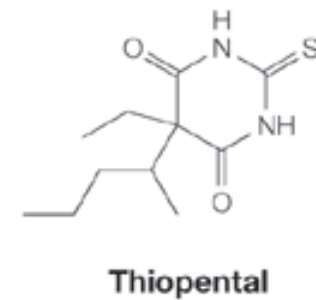
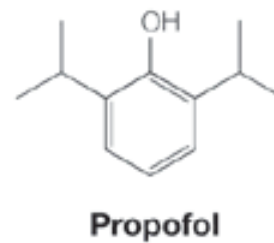
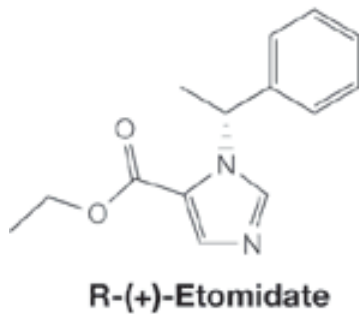
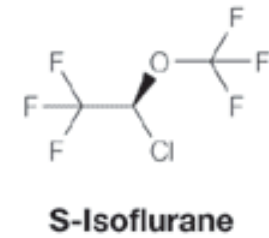
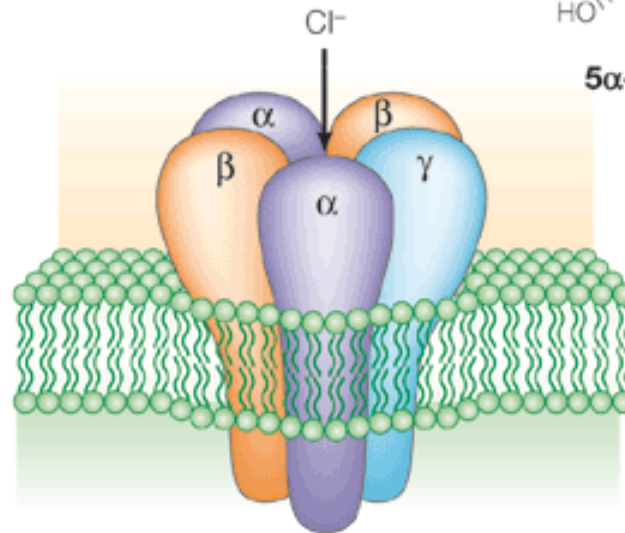
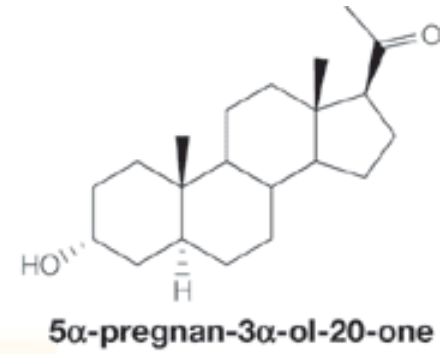
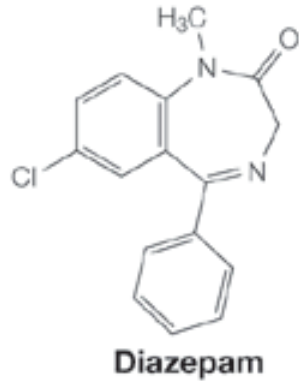
Benzodiazepines

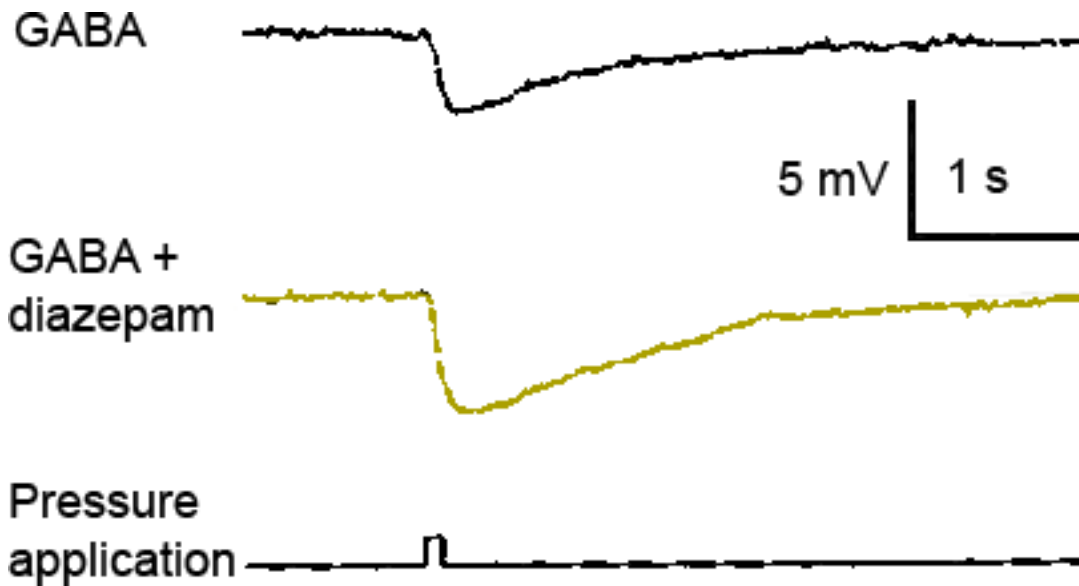
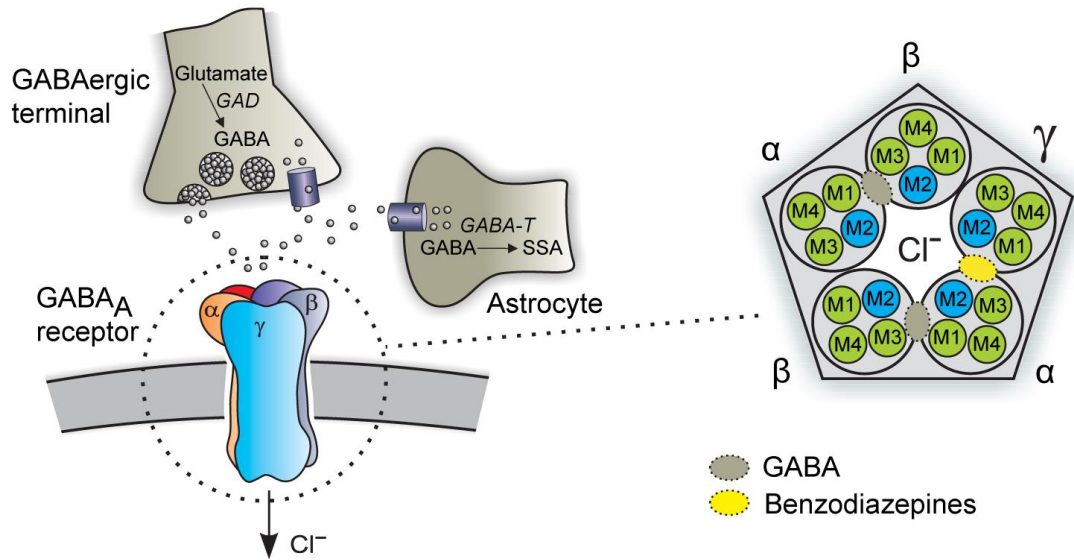
Benzodiazepines are the most widely used group of hypnotics and mediate their effects through allosteric binding and potentiation of GABA_A receptor-mediated chloride conductance. The majority of clinically used benzodiazepines bind with high affinity only to those receptors containing a γ 2 subunit, 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 α 1-, α 2-, α 3- or α 5-containing subtypes. Several benzodiazepine site ligands such as zolpidem and zaleplon demonstrate some binding selectivity for the α 1-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 α 1, α 2, α 3 and α 5 subunits that is crucial for benzodiazepine binding has led to the generation of mice with individual subtypes insensitive to diazepam [4,5,8]. α 1-containing receptors might contribute to sedation, [4,5] in line with the marked sedative properties of α 1-selective ligands. α 2 and α 3 subunits have been linked to anxiolytic effects [8,9], whereas α 5-containing receptors might play a role in cognitive liability [10].

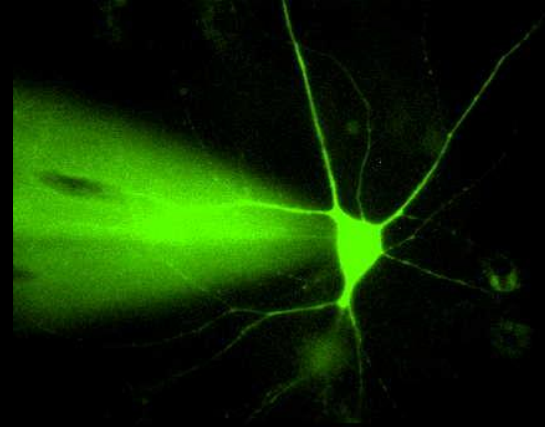
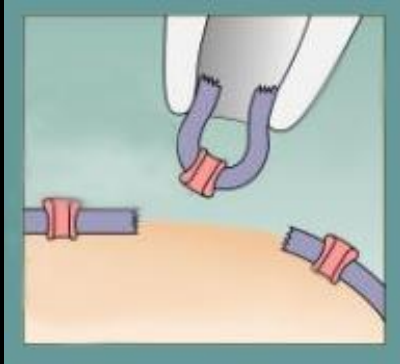
Limited or variable efficacy

Psychiatric side effects at supratherapeutic doses in abuse liability in drug abusers

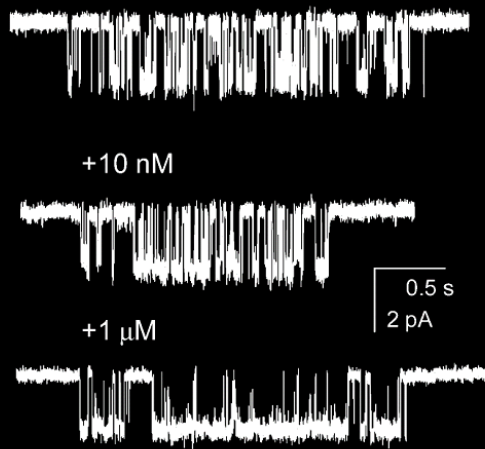




Single Channel Currents in Excised Outside-Out Patches from Recombinant GABA_A Receptor Transfected Cells



50 μ M GABA



$\alpha 1 \beta 1 \gamma 2 L$

GABA (1 μ M) + 5 α ,3 α -P (.1 μ M) + Pentobarb (50 μ M)



$\alpha 1 \beta 1$

