Table I. Classifications of antiepileptic drugs and mechanisms of action

Author(s), year of publication	Classifications of AEDs	
Ketter, 1999 [1] 2 categories	Predominantly GABA mechanisms of action	Anti-glutamatergic effects
	Typical: BBs, BZs, GPT, TGB, VGB, VPA, ZNS Partial: CBZ, OXC, PHT, TPM	Typical: FBM Partial: LTG
Rogawski & Loscher, 2004 [2] 3 categories	Predominantly sodium and calcium channel activity	GABA-mediated mechanisms Mixed, complex, or poorly understood actions
	CBZ, LTG, OXC, PHT, ZNS	BZs, TGB, VGB ESM, FBM, GPT, LEV, PB, TPM, VPA
Glauser, 2004 [3] 4 categories	Voltage-gated cationic ion channel modulation	Augmentation of GABAergic transmission Mixed GABAergic and antigultamateric actions Other than conventional actions
	CBZ, ESM, LTG, OXC, PHT, VPA, ZNS	BBs, BZs, GBP, TGB, VGB FBM, TPM LEV

AEDs: antiepileptic drugs; barbiturates (PB: phenobarbital; PRM: primidone); benzodiazepines (CLB: clobazam; CNZ: clonazepam); CBZ: carbamazepine; ESM: ethosuccimide; GBP: gabapentin; LEV: levetiracetam; LTG: lamotrigine; OXC: oxcarbazepine; PGB: pregabalin; PHT: phenytoin; VPA: valproic acid; TGB: tiagabine; VGB: vigabatrin; FBM: felbamate; TPM: topiramate; ZNS: zonisamide.

pharmacotherapy for epilepsy as the first AED of the modern era, Gowers stated that when attacks that have occurred for many years stop suddenly, whether the stoppage is spontaneous or due to the influence of drugs, patients may become dull, forgetful, and sometimes irritable or semi-idiotic.

In 1942, when phenobarbital (PB) was introduced in epilepsy treatment, Lennox [5] reported that occasional susceptible patients may suffer either in the direction of increased slowness or of active psychosis with medication. He reported that the incidence of mental worsening with PB was 11.6%, while that with bromides was 17.5%. In 1951, Gibbs [6] stated that psychosis seen as a complication of the treatment of psychomotor seizures with phenacemide has a mechanism similar to psychotic disorders which may occur when such seizures are suppressed by other anticonvulsant agents, such as barbiturates and hydantoins.

Consideration of the concept of forced normalization and its clinical counterpart, alternative psychosis, is essential when discussing the AED-related psychosis. In 1958, Landolt [7] reported productive psychotic episodes with "forced normalization" in the EEG. He wrote that this form of twilight state readily occurs on the institution of treatment, and that cautious discontinuation of medication frequently has a favorable effect in such cases. In 1965, Tellenbach [8] introduced the term "alternative psychosis" to refer to the clinical phenomenon of a reciprocal relationship between psychosis and seizures. Several psychopathological pictures have been linked to forced normalization, and psychosis is the most common [9].

Wolf *et al.* [9] reported that the incidence of forced normalization was 12 cases (15%) of 82 absence patients treated with ESM monotherapy, compared to none in 126 patients treated with valproate (VPA) monotherapy. Although ESM appears to be more likely to relate to

forced normalization, all AEDs can produce this phenomenon. Pakalnis *et al.* [10] reported seven psychotic cases with forced normalization, six with AED monotherapy (3 carbamazepine [CBZ], 2 methsuximide, and 1 VPA), and one with co-therapy (CBZ and VPA). With regards to newer AEDs, Trimble *et al.* [11] reported a retrospective case note study on 89 patients who developed psychosis or affective disorder during AED treatment, and concluded that forced normalization appeared to relate more to the use of GABAergic drugs, particularly with more powerful agents, such as topiramate (TPM), vigabatrin (VGB), and tiagabine (TGB).

Psychoses are often seen in a setting in which previously refractory patients suddenly become seizure-free. This phenomenon is not restricted to AED-induced seizure control. It is likely that in patients who develop *de novo* psychosis following epilepsy surgery, forced normalization may play such a role. A case of an alternative psychosis secondary to vagus nerve stimulation has been reported [12]. A rare case, which showed spontaneous seizure remission and the alternating emergence of psychotic symptoms, was reported in Japan [13].

Prevalence of AED-related psychosis

It is difficult to estimate the exact prevalence of AED-related psychosis. Because there are no systematic data with respect to older AEDs, retrospective clinical series and case reports should be included to estimate the prevalence of older AED-related psychosis. While many randomized controlled clinical trials for the newer AEDs are present, they are designed to test anti-seizure efficacy and have failed to use standardized psychiatric diagnostic criteria. Another problem regards the inconsistency of the definition of psychosis in the AED-related psychosis literature. Some reports have been based on standardized criteria for psychosis, such as the ICD-10 or DSM-IV, while others have adopted an in-house criterion of psychosis, and many others have used the term without definition. The follow-up should be long enough to differentiate between AED-related effects and the natural course of the comorbid disorder. An exact method to determine whether an AED is causing a psychosis would be to withdraw the drug, and subsequently rechallenge with it and observe the outcome. However, such studies have ethical limitations.

Although treatment emergent psychosis tends to be seen by all AEDs in those who are susceptible to developing psychosis, certain AEDs appear to be more likely to be associated with a psychosis. Kawasaki *et al.* [14] analyzed the association between AEDs and psychosis of epilepsy based on 26 Japanese patients, and concluded that the most relevant drugs were high-dose phenytoin (PHT) and zonisamide (ZNS). Cockerell *et al.* [15] surveyed the neurologists in the UK, and 19 cases were reported to have acute psychological disorders, including psychosis. They reported that VGB was the most commonly identified, followed by lamotrigine (LTG). Matsuura [16] analyzed 44 consecutive patients with epilepsy and acute psychosis, and reported that 17 cases (39%) were AED-related psychosis, and that adjunctive ZNS therapy was the most commonly attributed. Schmitz [17] analyzed 26 epilepsy patients with psychosis, and reported that four cases (16%) were AED-related, caused mainly by polytherapy with PHT.

Brodtkorb & Mula [18] recommended that VGB, TPM, ethosuccimide (ESM), and ZNS should be avoided in patients vulnerable to psychosis, and that levetiracetam (LEV) should be used with caution and benzodiazepines (BZD), gabapentin (GBP), and pregabalin (PGB) should be considered. According to the incidence of reported cases with AED-associated psychosis, three AED groups, frequently, moderately, and rarely associated with psychosis, might be distinguished.

AEDs frequently associated with psychosis *(Table II)*

Table II. AEDs frequently reported to induce psychosis (single case reports are omitted)

1. Suiccimides	
Fisher *et al.*, 1965 [19]	3 cases (2.9%) with 5 episodes of hallucinatory psychosis; disappeared after discontinuation of ESM
Wolf *et al.*, 1984 [9]	8 absence patients (3.5%) showed psychosis and forced normalization with ESM
Pakalnis *et al.*, 1987 [10]	2 TLE patients showed psychosis and forced normalization with methsuximide
2. TPM	
Crawford, 1998 [20]	11 cases (12%) showed psychotic symptoms with add-on therapy
Khan *et al.*, 1999 [21]	5 psychotic cases (0.8%) soon after starting the drug; symptoms resolved quickly after discontinuation or dose reduction
Trimble *et al.*, 2000 [11]	18 patients developed psychosis (5 post-ictal, 5 seizure suppression or reduction, 3 seizure increased, 5 seizure unchanged)
Stella *et al.*, 2002 [22]	2 cases with acute psychosis; full remission after interruption or reduction of dose
Kanner *et al.*, 2003 [23]	9 cases (1.5%) in a prospective multi-center study
Mula *et al.*, 2003 [24]	16 cases (3.7%) in a prospective study, mainly postictal psychosis, followed by alternative psychosis, and schizophrenia-like psychosis
Reith *et al.*, 2003 [25]	10 children and adolescents (6%) showed aggression or psychosis
Grosso *et al.*, 2005 [26]	6 children and adolescents (2%) showed acute psychosis in a study of long-term treatment
3. VGB	
Sander *et al.*, 1991 [27]	14 cases with intractable epilepsy with psychosis (including 4 post-ictal, 4 alternative, 1 overdose)
Cockerell *et al.*, 1996 [15]	4 cases with acute psychosis (3 with seizure improvement, 1 with seizure aggravation)
Thomas *et al.*, 1996 [28]	28 cases with psychosis, characterized by severe epilepsy, higher dose, right-sided focus, and seizure freedom
Trimble *et al.*, 2000 [11]	28 patients developed psychosis (including 7 post-ictal, 16 alternative)
4. ZNS	
Kawasaki *et al.*, 1991 [14]*	7 TLE cases with polytherapy; 5 experienced within 1 month, 3 were alternative psychosis
Matsuura *et al.*, 1993 [29]*	8 cases (6 TLE, 2 FLE) with polytherapy; 3 were alternative psychosis and 2 post-ictal psychosis
Hara *et al.*, 1993 [30]*	5 TLE cases with polytherapy; average latency of psychosis was 6 months, 3 were alternative psychosis
Mayahara *et al.*, 1995 [31]*	3 TLE cases with alternative psychosis, one with monotherapy
Miyamoto *et al.*, 2000 [32]	14 cases (19%) in a retrospective study; 7 developed psychosis within 1 year, alternative factors may have contributed

* In Japanese.
FLE: frontal lobe epilepsy; TLE: temporal lobe epilepsy.

Succinamides: ethosuccimide and methsuximide

ESM modifies the properties of voltage-dependent calcium channels, reducing T-type currents and thereby preventing synchronized firing. Fisher *et al.* [19] reported that three patients (2.8%) in 105 epileptic patients developed five psychotic episodes occurring within a few days of ESM treatment. The EEG often reverted to normal during the psychotic episodes. Because the psychotic symptoms improved after ESM discontinuation, they considered the symptoms as exogenous psychosis. Wolf *et al.* [9] reported that 18 cases (7.8%) showed forced normalization in 229 absence patients treated with ESM mono/polytherapy, and that eight (3.5%) of these patients showed paranoid episodes. Pakalnis *et al.* [10] described two patients with complex partial seizures whose psychotic symptoms emerged shortly after starting methsuximides. The temporal lobe abnormalities present on the EEGs prior to treatment became normalized during the patients' psychotic episodes.

Topiramate

TPM was introduced into the Japanese market in 2007 for adjunctive therapy in treating partial seizures. It has a wide range of potentially anticonvulsant actions, such as an antiglutamatergic action at AMPA/kainate receptors, blockage of voltage dependent sodium channels, potentiation of GABAergic inhibition, and carbonic anhydrase inhibition. Crawford [20] carried out an audit of TPM use at a general neurology clinic, and 11 cases (12%) of 94 patients were identified as having psychotic symptoms, which was significantly higher than that for patients treated with other AEDs. Khan *et al.* [21] reported that five patients (0.8%) showed psychotic symptoms soon after beginning TPM with the dose of 50-400 mg, and that these symptoms resolved quickly after discontinuation or dose reduction of TPM. Trimble *et al.* [11] reported 18 cases with TPM-related psychosis, and stated that the low dose group developed with a shorter interval between the start of the TPM therapy and the onset of psychosis, compared with the high dose groups. Kanner *et al.* [23] conducted a prospective multi-center study, and reported that the prevalence of TPM-induced psychosis in polytherapy regimens was 9 cases (1.5%) of 596 patients, and that patients with a past psychiatric history present a higher risk of experiencing psychiatric as well as cognitive adverse events. Mula *et al.* [24] conducted a prospective study of 431 patients treated with TPM to determine the prevalence of psychiatric events. Sixteen patients (3.7?) were reported to have had psychotic disorders, mainly postictal psychosis (1.7%), followed by alternative psychosis (1.1%), and schizophrenia-like psychosis (0.9%). They concluded that the risk factors were high starting dose, rapid titration, a psychiatric history, and more severe epilepsy with a high seizure frequency.

Reith *et al.* [25] conducted a retrospective cohort study of 159 patients aged less than 18 years, and reported that 10 cases (6%) developed aggression or psychosis, especially in those with a history of prior aggression. Grosso *et al.* [26] reported that acute psychosis occurred in 6 patients (2%) in a study of the long-term treatment of 277 children and adolescents. Therefore, TPM may itself induce psychosis in susceptible patients, and slow dose titration may reduce the risk of this side effect.

Vigabatrin (VGB)

VGB is a structural analogue of GABA, which exerts its anticonvulsant action by irreversibly binding to GABA transaminase, blocking degradation of GABA and increasing its concentration in the brain. It was never approved for use in Japan, due to

evidence of irreversible peripheral visual field loss in a portion of patients with chronic use. Sander *et al.* [27] reported a series of 14 cases of psychosis occurring in patients with severe intractable epilepsy, following the prescription of VGB with a polytherapy regimen. Four developed psychosis after seizure freedom (alternative psychosis), while the other four developed after a period of seizure freedom followed by a cluster of seizures (postictal psychosis). The period to the onset of psychosis was 5 days to 32 weeks, and all resolved on withdrawal. They stated that VGB had powerful antiepileptic action and should be started with caution in patients with a previous history of psychosis. Ferrie *et al.* [33] reviewed seven placebo-controlled European studies and showed an overall occurrence of psychosis of 3.4% in the VGB group and 0.6% in the placebo group. Levinson & Devinsky [34] conducted another meta-analysis of double-blind studies, and demonstrated that psychosis occurred 2.5% in the VGB group compared to 0.3% in the placebo group. Thomas *et al.* [28] reported 28 cases with VGB-treatment emergent psychosis, and concluded that the risk factors were severe epilepsy, right-sided focus, suppression of seizures, and a history of psychosis compared with the VBG-induced depression group. Psychosis may be caused by a direct pharmacological action of VGB, or through indirect mechanisms which may arise from its effects on seizure control.

Zonisamide

ZNS has been approved since 1987 in Japan for partial epilepsy. It has a number of different effects in the brain which may be responsible for its anticonvulsant effects, including blockage of T-type calcium channels, inhibition of sodium channels, possible inhibition of glutamate release, and also mild inhibition of carbonic anhydrase. Matsuura & Trimble [35] reviewed five Japanese papers on ZNS-associated psychosis, and estimated the prevalence at 2%. Miyamoto *et al.* [32] identified 14 epileptic patients (19%) with psychotic episodes in a total of 74 young patients, in a retrospective study on ZNS treatment over a 10-year period. Because 7 patients developed psychosis a few years after the initiation of ZNS treatment, they stated that alternative factors may have caused or contributed to the development of psychosis. ZNS enhances dopamine transmission and was approved for use in Parkinson disease in 2008 in Japan. Its dopamine effect may also contribute to the induction of psychosis in epilepsy.

AEDs moderately associated with psychosis *(Table III)*

Table III. AEDs sometimes reported to induce psychosis (single case reports are omitted)

1. Barbiturates, benzodiazepines	
Demers-Desrosiers *et al.*, 1978 [36]	2 cases with psychotic symptoms immediately after withdrawal of AEDs including PB and PRM
Kugoh *et al.*, 1990 [37]	2 psychotic cases with toxic PB serum levels
Sironi *et al.*, 1979 [38]	2 cases with acute psychosis as a CZP withdrawal syndrome
Hauser *et al.*, 1989 [39]	3 cases with BZ withdrawal delirium with catatonic features
2. LTG	
Crawford, 1998 [20]	2 psychotic cases (0.7%) with add-on therapy

Clemens, 2005 [40]	2 psychotic cases (a child with BECT and an adult with TLE) with forced normalization
Brandt *et al.*, 2007 [41]	6 cases (0.4%) with schizophrenia-like psychosis; risk factors are high dose, temporal lobe pathology, and past psychiatric history
3. LEV	
Kossoff *et al.*, 2001 [42]	1 child and 3 adolescent cases with psychosis; all had previous behavior problems or cognitive deficit
Mula *et al.*, 2003 [43]	6 psychotic cases (1.2%) in a prospective study
4. PHT	
Franks & Richter, 1979 [44]	2 cases with psychotic symptoms as an organic brain syndrome
Kawasaki *et al.*, 1991 [14]*	4 TLE cases with psychosis (3 were alternative psychosis with high-dose monotherapy)
Sengoku *et al.*, 1991 [45]*	2 TLE cases of alternative psychosis with high-dose monotherapy
Watanabe *et al.*, 1994 [46]*	4 alternative psychosis cases with high-dose therapy
5. TGB	
Cockerell *et al.*, 1996 [4]	2 cases with alternative paranoid psychosis
Trimble *et al.*, 2000 [11]	3 cases with psychosis
Sackellares *et al.*, 2002 [47]	3 cases (0.8%) with psychotic symptoms with add-on therapy, in a multi-center, double-blind, placebo-controlled trial

* In Japanese.
BECT: benign epilepsy of children with centro-temporal spikes; TLE: temporal lobe epilepsy.

Barbiturates: phenobarbital and primidone

The anticonvulsant action of Barbiturates is probably related to their enhancement of GABA-mediated inhibition. Twenty to 40% of children being treated with PB developed a behavior disorder, such as hyperactivity, depression, irritability, and aggressiveness [48]. In adults, a high dose of PB could induce psychosis [37]. Primidone (PRM) is also reported to produce acute psychotic symptoms, including bizarre and hallucinatory behavior immediately after administration [48]. Demers-Desrosiers *et al.* [36] reported two cases of patients with epilepsy who became psychotic immediately after withdrawal of AEDs, including PB and PRM. These psychoses occurred in the context of clear sensorium, accompanied by markedly increased EEG abnormalities, and recovered completely within days after the reinstitution of medication. Kanner *et al.* [49] have emphasized that interictal psychiatric symptoms worsened in severity during the postictal period, and that 19 patients taking one AED with negative psychotropic properties (PB, PRM, or VGB) yielded a trend towards a greater likelihood of developing postictal psychosis.

Benzodiazepines

Benzodiazepines and barbiturates enhance GABAergic inhibition by interacting directly with GABA-A receptors. Withdrawal syndrome with prominent psychiatric symptoms including psychosis can occur with barbiturates and benzodiazepines. Sironi *et al.* [38]

reported two patients with temporal lobe epilepsy and acute psychosis manifesting as CZP withdrawal syndrome. They stated that the patents' psychomotor seizures disappeared, and depth EEG recording showed a marked reduction of repetitive abnormalities during the psychotic state (forced normalization). Hauser *et al.* [39] reported three cases with delirium and catatonic symptoms as benzodiazepine withdrawal syndrome (clorazepate dispotassium, 2 patients; CZP, 1 patient). On the other hand, Franks & Richter [44] reported three cases with schizophrenia-like psychosis associated with AED toxicity, and one of these developed delusional and hallucinatory behavior after an overdose of CZP.

Lamotrigine

LTG is a generally well-tolerated drug with broad-spectrum efficacy and was approved as an adjunctive treatment for partial epilepsy in 2008 in Japan. LTG probably exerts its anticonvulsant effects *via* a combination of sodium channel inhibition and calcium channel effects. Sporadic case reports have appeared of psychosis attributed to LTG treatment. The incidence of psychotic symptoms in 270 patients taking LTG in a general neurology clinic was 0.7% [20]. Clemens [40] reported two cases with LTG-induced forced normalization, and a reduction of LTG led to the disappearance of the symptoms and the reappearance of spikes on their EEGs. Brandt *et al.* [41] reported six cases with schizophrenia-like psychotic disorders, the symptoms of which improved rapidly after discontinuing LTG or decreasing the dose. They reported that one patient who was re-exposed to LTG again presented similar symptoms.

Levetiracetam

LEV has a specific CNS-limited binding site unique among AEDs, and its exact mechanism is unknown. Although the drug is generally well tolerated, behavioral side effects have been reported with variable frequency. Mula *et al.* [43] prospectively studied psychiatric adverse events during LEV therapy, and reported psychiatric adverse events in 53 cases (10.1%) from 517 patients, among which were 6 cases (1.2%) with psychosis. Cramer *et al.* [50] conducted a meta-analysis, and reported that the prevalence of psychosis was 1.4%. Regarding children and adolescents, Kossoff *et al.* [42] reported one child and three adolescents with LEV treatment-emergent frank psychosis. All of these patients had behavior problems or cognitive deficits before initiating LEV, and all experienced dramatic improvement within days of either discontinuing or decreasing the dose of LEV.

Phenytoin

The anticonvulsant effect of PHT is related to sodium channel blockade. Several anecdotal case reports have appeared of high dose PHT-related psychosis. Franks & Richter [44] reported two cases with psychotic symptoms characterized by clear sensorium with minimal signs of toxicity, classed as PHT organic brain syndrome. Kawasaki *et al.* [14] reported four cases of temporal lobe epilepsy which developed PHT-related psychosis, three of which were alternative psychosis with high-dose monotherapy. Watanabe *et al.* [46] reported four psychotic cases with PHT high-dose treatment. All of these patients exhibited schizophrenia-like psychosis with clear consciousness and epileptiform discharges on their EEGs.

Tiagabine

TGB inhibits neuronal and glial GABA reuptake, thereby enhancing GABA's inhibitory effect. Because TGB has a mechanism of action similar to that of VGB, concern has been raised regarding its potential to cause treatment-emergent psychosis. Sackellares *et al.* [47] conduced an ad hoc analysis of two multi-center, randomized, double-blind, placebo-controlled studies of add-on therapy of TGB, and 3 cases (0.8%) of 356 TGB-treated patients developed psychosis, compared to none of 198 placebo-treated patients. Cockerell *et al.* [15] reported two psychotic cases. Trimble *et al.* [11] reported three cases with TGB-related psychosis.

AEDs rarely associated with psychosis *(Table IV)*

Table IV. AEDs rarely reported to induce psychosis (single case reports are included)

1. CBZ	
Franks & Richter, 1979 [44]	1 case with psychotic symptoms exacerbated by rechallenge
Pakalnis *et al.*, 1987 [10]	4 psychotic cases with forced normalization (3 monotherapy and 1 co-therapy with VPA)
Mathew, 1988 [51]	1 case with epilepsy and mild mental handicap; psychosis developed shortly after change from VPA
McKee *et al.*, 1989 [52]	1 case with acute psychotic reaction shortly after add-on therapy
Samuimi-Ardestani *et al.*, 2008 [53]	1 TLE case with hallucinatory symptom; disappeared with discontinuation
2. FBM	
Knable & Kenneth, 1995 [54]	1 case with long-standing hypoxic brain damage developed severe psychotic symptoms
McConnell *et al.*, 1996 [55]	1 case with psychosis
3. GBP/PGB	
Crawford, 1998 [20]	1 case (0.5%) with GBP add-on therapy
Olaizola *et al.*, 2006 [56]	1 case with psychotic symptoms with PGB
4. VP	
Pakalnis *et al.*, 1987 [10]	2 psychotic cases with forced normalization (1 absence patient with monotherapy, 1 TLE patient with co-therapy with CBZ)

TLE: temporal lobe epilepsy

Carbamazepine and oxcarbazepine

These AEDs probably exert their anticonvulsant effects by sodium channel blockade, though they also have other sites of action. CBZ is approved for use in Japan for the manic state an excited state of schizophrenia. On the other hand, its prescribing information designates that the drug can provoke hallucinations and/or excitations. Rare but sporadic epilepsy cases with CBZ-related psychosis were reported as a direct side effect [44, 51-53] or forced normalization [10].

Although no previous studies have reported psychosis as a side effect of OXC, a case with Parkinson disease which developed psychotic symptoms, probably through the dopamine agonistic mechanism of OXC, was reported [57].

FBM

FBM has a number of anti-excitatory effects, which account for its anticonvulsant effects, including that on NMDA and non-NMDA excitatory amino acid receptors, as well as the inhibition of voltage-gated sodium channels. It is rarely used at present, due to serious hepatic and hematological adverse effects in some patients. Rare but sporadic epileptic patients with treatment related psychosis have been reported when receiving FEL monotherapy [55] or FBM add-on therapy [54].

Gabapentin and pregabalin

Despite being analogs of GABA, the anticonvulsant actions of GBP and PGL are likely to not be related to effects on the usual GABA binding sites. Their mechanism of action remains unknown. In the audit of the use of AEDs in a general neurology clinic carried out by Crawford [20], one case (0.5%) of 191 patients receiving GPT add-on therapy exhibited psychotic symptoms. A 44-year-old female with acute psychosis associated with marked EEG exacerbation after rapid titration of a relatively large dose of PGL was also reported [56].

Valproate

The mechanism of action of VPA may include the potentiation of GABAergic functions and inhibition of voltage-sensitive sodium channels. VPA is approved for use in Japan for the manic state and behavior disorders of epilepsy, such as dysphoria and/or aggression. Pakalnis *et al.* [10] reported two psychotic cases; one with VPA monotherapy, and one with cotherapy with VPA and CBZ. They considered these to be induced by forced normalization and not as a direct effect of VPA.

Mechanisms of AED-induced psychosis

Ketter [1] has classified AEDs into those with predominantly GABA mechanisms of action and those with antiglutamatergic effects *(Table 1)*. Rogawski & Loscher [2] have categorized AEDs into three categories: (1) predominant sodium (and calcium) channel activity; (2) GABA-mediated mechanisms; and (3) mixed, complex or poorly understood actions *(Table 1)*. Glauser [3] has grouped AEDs into four broad categories based on their major mechanisms of action: (1) voltage-gated cationic ion channel modulation; (2) augmentation of GABAergic transmission; (3) mixed GABAergic and antigultamateric actions; and (4) other than conventional actions *(Table 1)*. All of these classifications of AEDs do not correlate with the rate of treatment-emergent psychosis, and AED-related psychosis seems to occur irrespective of the mechanisms of action of the AED.

Matsuura [16] reported 17 patients with AED-related psychosis, including seven following rapid titration, six after acute discontinuation, and four after taking an overdose of AEDs. The follow-up study revealed that six showed recurrent psychosis without a clear relationship with any AED, and one showed a chronic course of psychosis. Schmidt *et al.* [17] analyzed 26 epileptic patients with AED-related psychosis, and reported that 8% were alternative, 4% withdrawal, and 4% intoxication from AEDs. Weintraub *et al.* [58]

reported that the average rate of AED-related psychopathology for a single AED was 8.4%, with 6.1% resulting in dose change and 4.3% resulting in AED discontinuation. It appears that psychoses with the newer AEDs occurred frequently in early clinical trials, involving a dosing schedule that subsequently appeared to be rapid, or doses that were too high. Because rapid changes in the regimen of powerful AEDs induce psychosis, it can be argued that the underlying pathomechanisms are common. A dramatic alteration in the balance between inhibitory and excitatory processes, a deficit of homeostasis in the brain, may play a key role in AED-related psychosis.

Conclusion

A selective review of the published literature in English and Japanese on AED-related psychosis was carried out. All AEDs can induce treatment-induced psychosis, regardless of the mechanisms of action, and it can be argued that the underlying pathomechanism is common. Because rapid changes in the regimen of powerful AEDs induce acute psychosis, a dramatic alteration in the balance between inhibitory and excitatory processes may play a key role. AED-induced psychosis is typically transient and responsive to a reduction or discontinuation of the drug or to antipsychotic treatment. Although it may be rare, psychosis can reoccur without relating to medication or persist chronically. When prolonged overinhibition persists, recurrent or chronic psychosis may occur. Powerful AEDs should be used with a slow titration schedule and with monotherapy, especially those prone to develop psychosis.

References *[[To the author: please check ref. 8]]*

1. Ketter TA, Post RM, Theodore WH. Positive and negative psychiatric effects of antiepileptic drugs in patients with seizure disorders. *Neurology* 1999; 53 (suppl 2): S53-67.
2. Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. *Nature* 2004; 5: 553-64.
3. Glauser TA. Effects of antiepileptic medications on psychiatric and behavioral comobidities in children and adolescents with epilepsy. *Epilepsy Behav* 2004; 5: S25-S32.
4. Schmidt B. Forced normalization: History of a concept. In: Trimble MR, Schmitz B, eds. *Forced Normalization and Alternative Psychoses of Epilepsy*. Petersfield: Wrightson Biomed Publ Ltd, 1998: 7-24.
5. Lennox WG. Brain injury, drugs, and environment as causes of mental decay in epilepsy. *Am J Psychiatry* 1942; 99: 174-80.
6. Gibbs FA. Ictal and non-ictal psychiatric disorders in temporal lobe epilepsy. *J Nerv Ment Dis* 1951; 113: 522-8.
7. Landolt H. Serial electroencephalographic investigation during psychotic episodes in epileptic patients and during schizophrenic attacks. In: Lorenz de Haes AM, ed. *Lectures on Epilepsy*. Amsterdam: Elsevier, 1958: 91-133.
8. Tellenbach H. Epilepsie als Anfallsleiden und als Psychose. U"ber alternative Psychosen paranoider Pra"gung bei "forcierter Normalisierung" (Landolt) des Elektroenzephalogramms Epileptischer. *Nervenarzt* 1965; 36: 190-202.
9. Wolf P, Inoue Y, Röder-Wanner U, *et al*. Psychiatric complications of absence therapy and their relation to alteration of sleep. *Epilepsia* 1984; 25: S56-S59.
10. Pakalnis A, Drake ME, John K, *et al*. Forced normalization: Acute psychosis after seizure control in seven patients. *Arch Neurol* 1987; 44: 289-92.

11. Trimble MR, Rusch N, Betts T, *et al*. Psychiatric symptoms after therapy with new antiepileptic drugs: psychopathological and seizure related variables. *Seizure* 2000; 9: 249-54.
12. Gatzonis SD, Stamboulis E, Siafakas A, *et al*. Acute psychosis and EEG normalization after vagus nerve stimulation. *J Neurol Neurosurg Psychiatry* 2000; 69: 278-9.
13. Minabe Y, Kurachi M: A case of epilepsy with "forced normalization" without antiepileptic treatment. *Seishin-Igaku* 1990; 32: 196-8.
14. Kawasaki J, Sengoku A, Kanemoto K, *et al*. Two occurrences of acute paranoid-hallucinatory state in epilepsy; in association with antiepileptic drugs. *Seishin-Igaku* 1991; 32: 595-600.
15. Cockerell OC, Moriatry J, Trimble M, *et al*. Acute psychological disorders in patients with epilepsy: a nation-wide study. *Epilepsy Res* 1996; 25: 119-31.
16. Matsuura M. Epileptic psychoses and anticonvulsant drug treatment. *Journal of Neurology, Neurosurgery and Psychiatry* 1999; 67: 231-3.
17. Schmitz B, Robertson MM, Trimble MR. Depression and schizophrenia in epilepsy: social and biological risk factors. *Epilepsy Res* 1999; 35: 59-68.
18. Brodtkorb E, Mula M. Optimizing therapy of seizures in adult patients with psychiatric comorbidity. *Neurology* 2006; 67 (suppl 4): S39-S44.
19. Fisher M, Korskjaer G, Pedersen E. Psychotic episodes in Zarondan treatment. *Epilepsia* 1965; 6: 325-34.
20. Crawford P. An audit of topiramate use in a general neurology clinic. *Seizure* 1998; 7: 207-11.
21. Khan A, Faught E, Gilliam F, *et al*. Acute psychotic symptoms induced by topiramate. *Seizure* 1999; 8: 235-7.
22. Stella F, Caetano D, Cendes F, *et al*. Acute psychotic disorders induced by topiramate. *Arq Neuropsiquiatr* 2002; 60: 285-7.
23. Kanner AM, Wuu J, Faught E, *et al*. A past psychiatric history may be a risk factor for topiramate-related psychiatric and cognitive adverse events. *Epilepsy Behav* 2003; 4: 548-52.
24. Mula M, Trimble MR, Lhatoo SD, *et al*. Topiramate and psychiatric adverse events in patients with epilepsy. *Epilepsia* 2003; 44: 659-63.
25. Reith D, Burke C, Appleton DB, *et al*. Torelability of topiramate in children and adolescents. *J Paediatr Child Health* 2003; 39: 416-9.
26. Grosso S, Franzoni E, Iannetti P, *et al*. Efficacy and safety of topiramate in refractory epilepsy of childhood: long-term follow-up study. *J Child Neurol* 2005; 20: 893-7.
27. Sander JW, Hart YM, Trimble MR, *et al*. Vigabatrin and psychosis. *J Neurol Neurosurg Psychiatry* 1991; 54; 435-9.
28. Thomas L, Trimble MR, Schmitz B, *et al*. Vigabatrin and behaviour disorders: a retrospective survey. *Epilepsy Res* 1996; 25: 21-7.
29. Matsuura M, Senzaki A, Okubo Y, *et al*. Eight epileptic patients showing delusional-hallucinatory state during zonisamide administration. *Seishin-Igaku* 1993; 35: 413-9.
30. Hara S, Imasaka Y, Takei S, *et al*. Five cases of zonisamide-related psychosis: with particular reference to the drug interaction between zonisamide and phenytoin and to the risk factors for epileptic psychosis. *Seishinka-Chiryogaku* 1993; 8: 59-64.
31. Mayahara K, Kanemoto K, Kawasaki J, *et al*. Zonisamide and epileptic psychosis. *Tenkan-Kenkyu* 1995; 13: 177-83.
32. Miyamoto T, Kohsaka M, Koyama T. Psychotic episodes during zonisamide treatment. *Seizure* 2000; 9: 65-70.
33. Ferrie CD, Robinson RO, Panayiotopoulos CP. Psychiatric and severe behavioural reactions with vigabatrin: a review. *Acta Neuro Scand* 1996; 93: 1-8.
34. Levinson DF, Devinsky O: Psychiatric adverse events during vigabatrin therapy. *Neurology* 1999; 53: 1503-11.

35. Matsuura M, Trimble MR: Zonisamide and psychoses. *J Epilepsy* 1997; 10: 52-4.
36. Demers-Desrosiers LA, Nestoros JN, Vaillancourt P. Acute psychosis precipitated by withdrawal of anticonvulsant medication. *Am J Psychiatry* 1978; 135: 981-3.
37. Kugoh T, Inoue T, Utsumi T, *et al*. Five epileptic cases who manifested psychotic states under the toxic levels of antiepileptic drugs. *Jpn J Psychiat Neurol* 1990; 44: 392-3.
38. Sironi VA, Franzini A, Ravagnati L, *et al*. Interictal acute psychosis in temporal lobe epilepsy during withdrawal of anticonvulsant therapy. *J Neurol Neurosurg Psychiatry* 1979; 42: 724-30.
39. Hauser P, Devinsky O, De Bellis M, *et al*. Benzodiazepine withdrawal delirium with catatonic features. *Arch Neurol* 1989; 46: 696-9.
40. Clemens B. Forced normalization precipitated by lamotrigine. *Seizure* 2005; 14: 485-9.
41. Brandt C, Fueratsch N, Boehme V, *et al*. Development of psychosis in patients with epilepsy treated with lamotrigine: report of six cases and a review of literature. *Epilepsy Behav* 2007; 11: 133-9.
42. Kossoff EH, Bergey GK, Freeman JM, *et al*. Levetiracetam psychosis in children with epilepsy. *Epilepsia* 2001; 42: 1611-3.
43. Mula M, Trimble MR, Yuen A, *et al*. Psychiatric adverse events during levetiracetam therapy. *Neurology* 2003; 61: 704-6.
44. Franks RD, Richter AJ: Schizophrenia-like psychosis associated with anticonvulsant toxicity. *Am J Psychiatry* 1979; 136: 973-4.
45. Sengoku A, Kawasaki J, Kanemoto K, *et al*. High-dose monotherapy of phenytoin for intractable temporal lobe epilepsy. *Tenkan-Kenkyu* 1991; 9: 117-23.
46. Watanabe M, Kugoh T, Akada K, *et al*. High serum level therapy of phenytoin in epilepsy. *Seishinka-Chiryogaku* 1994; 9: 729-36.
47. Sackellares JC, Krauss G, Sommerville KW, *et al*. Occurrence of psychosis in patients with epilepsy randomized to tiagabine or placebo treatment. *Epilepsia* 2002; 43: 394-8.
48. Vining EPG. Use of barbiturates and benzodiazepines in treatment of epilepsy. *Neurol Clin* 1986; 4: 617-32.
49. Kanner AM, Soto A, Gross-Kanner H. Prevalence and clinical characteristics of postictal psychiatric symptoms in partial epilepsy. *Neurology* 2004; 62: 708-13.
50. Cramer JA, de Rue K, Devinsky O, *et al*. A systematic review of the behavioral effects of levetiracetam in adults with epilepsy, cognitive disorders, or an anxiety disorder during clinical trials. *Epilepsy Behav* 2003; 4: 124-32.
51. Mathew G. Psychiatric symptoms associated with carbamazepine. *BMJ* 1988; 296: 1071.
52. McKee RJW, Larkin JG, Brodie MJ. Acute psychosis with carbamazepine and sodium valproate. *Lancet Jan* 1989; 21: 167.
53. Samuimi-Ardestani SM, Zahiroddin A, Khodaifar F, *et al*. Epilepsy presenting panic attacks and hallucinatory experiences as drug side effect: a case report. *Ann Gen Psychiatry* 2008; 7: S119.
54. Knable MB, Rickler K. Psychosis associated with felbamate treatment. *J Clinical Psycho* 1995; 15: 292-3.
55. McConnell H, Snyder PJ, Duffy JD, *et al*. Neuropsychiatric side effects related to treatment with felbamate. *J Neuropsychiatry Clin Neurosci* 1996; 8: 341-6.
56. Olaizola I, Ellger T, Young P, *et al*. Pregabalin-associated acute psychosis and epileptiform EEG-changes. *Seizure* 2006; 15: 208-10.
57. Kovacs N, Nagy F, Balas I, *et al*. Oxcarbazepine may induce psychotic symptoms in Parkinson's disease. *Epilepsy Behav* 2008; 12: 492-3.
58. Weintraub D, Buchsbaum R, Resor SR, *et al*. Psychiatric and behavioral side effects of the newer antiepileptic drugs in adults with epilepsy. *Epilepsy Behav* 2007; 10: 105-10.

Behavioural Brain Research 207 (2010) 153–154

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Alpha-fluoromethylhistidine, a histamine synthesis inhibitor, inhibits orexin-induced wakefulness in rats

Seki Yasuko[a], Akanmu Moses Atanda[a,2], Matsuura Masato[b], Yanai Kazuhiko[c], Honda Kazuki[a,b,*,1]

[a] Department of Biosystem Regulation, Tokyo Medical and Dental University, Tokyo, Japan
[b] Department of Life Sciences and Bio-informatics, Tokyo Medical and Dental University, Tokyo, Japan
[c] Department of Pharmacology, Tohoku University School of Medicine, Sendai, Japan

ARTICLE INFO

Article history:
Received 11 May 2009
Received in revised form 6 September 2009
Accepted 30 September 2009
Available online 8 October 2009

Keywords:
Orexin
α-FMH
Histamine
REM sleep
NREM sleep
Wake
Rats

ABSTRACT

Orexins A and B are involved in the regulation of feeding and arousal state. Previously, we reported that third intracerebroventricular (icv) infusion of both orexins A and B induced a significant arousal effect in rats. We determined the effects of intraperitoneal (i.p.) injection of alpha-fluoromethylhistidine (α-FMH), a histamine synthesis inhibitor, on orexin-induced wakefulness in freely behaving rats. Male Sprague–Dawley rats were chronically implanted with cortical electroencephalogram (EEG) and neck electromyogram (EMG) electrodes, and a cannula for icv infusion. EEG and EMG were monitored for three consecutive days during continuous icv saline infusion at a rate of 10 μl/h. For a 5-h diurnal period, orexin-B (10 nmol/50 μl saline) replaced the icv infusion of saline. α-FMH (100 mg/kg, i.p.) was administered 6 h before icv infusion of orexin-B.

Orexin-B at a dose of 10 nmol/h markedly increased the amount of wakefulness by 99.4% ($p < 0.05$) over the baseline value, whereas α-FMH decreased orexin-B-induced wakefulness by 48.8%. Orexin-B-induced suppression of non-REM sleep was reversed by α-FMH treatment. Pretreatment with α-FMH, significantly inhibited orexin-B-induced wakefulness in rats. The findings of this study therefore suggest that arousal-state regulation by orexin neurons is possibly mediated via the histaminergic system in the tuberomammilary nucleus.

1. Introduction

Orexin receptors are broadly expressed in the central nervous system (CNS) and are reported to play an important role in regulating and maintaining sleep–wakefulness states and energy homeostasis. A previous report showed that when rats were treated with saponin–orexin conjugates to selectively produce lesions in orexinergic neurons in the lateral hypothalamus, a marked decrease in locomotor activity was observed with hypersomnolence, in addition to some features of narcolepsy [7]. Similarly, mice lacking orexin peptide were reported to display an increase in rapid eye movement (REM) and non-REM sleep with a decrease in wake activity time during the active period [4]. Further study also showed that canine narcolepsy is caused by a mutation in the orexin-2-receptor gene [9]. The importance of orexin-B in promoting the arousal state is clearly demonstrated in all of these studies.

Orexin-containing cells project widely throughout the entire neuroaxis [20]; their abundant projections are found particularly in monoaminergic cell groups that include histaminergic cells of the tuberomammilary nucleus (TMN) [4,14], where orexin-2-receptors are highly abundant [10]. Previously, we reported that intracerebroventricular (icv) infusion of both orexins A and B induced a significant arousal effect in rats [2]. Histamine-containing neurons of the TMN have been implicated in facilitating wakefulness [15]. Yamanaka et al. reported that orexins activate histaminergic neurons via the orexin-2-receptor in rats and showed that immunohistochemical and electron microscopic techniques revealed direct synaptic interaction between orexin-immunoreactive neurons in the TMN [21]. Alpha-fluoromethylhistidine, a histamine synthesis inhibitor, is reported to significantly decrease brain histamine content and locomotor activity after intraperitoneal administration [13,16,17]. Therefore, in the present study we investigated the effects of intraperitoneal (i.p.) injection of alpha-fluoromethylhistidine (α-FMH), a histamine synthesis inhibitor [11,18], on orexin-B induced wakefulness in rats.

* Corresponding author at: Sleep Science Laboratories, Tsukuba Research Center, Hamri CO., LTD 696-1 Kamikatata, Koga-shi, Ibaraki 306-0128, Japan. Tel.: +81 280 75 2511; fax: +81 280 76 6665.
E-mail address: kazhonda@hamri.co.jp (H. Kazuki).
[1] Present address: Sleep Science Laboratories, Tsukuba Research Center, Hamri CO., LTD 696-1 Kamikatata, Koga-shi, Ibaraki 306-0128, Japan.
[2] Present address: Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

0166-4328/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2009.09.049

2. Materials and methods

2.1. Animals

Between 16 and 18 male Sprague–Dawley rats (260–300 g) obtained from Crea Japan, Inc. (Tokyo, Japan) were housed in cages for 1 week. The room was maintained at a temperature of 25 ± 1 °C, relative humidity of 54 ± 6%, and a light–dark cycle of 12:12 h (lights on at 8:00 h). All experimental protocols were performed in accordance with the Guidelines for Animal Experimentation of Tokyo Medical and Dental University.

2.2. Preparation of orexin-B and α-FMH

Orexin-B (Peptide Institute, Inc., Osaka, Japan) and α-FMH (Merck Sharp & Dohme Research Laboratory, Rahway, NJ, USA) were dissolved in normal saline solution. Orexin-B or vehicle was continuously infused icv at a rate of 10 μl/h while α-FMH was administered intraperitoneally (i.p.) for the analysis of sleep–wake states.

2.3. Electroencephalogram (EEG) and electromyogram (EMG)

Male Sprague–Dawley rats (60–70 days old) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus; EEG and EMG recordings were performed simultaneously as described previously [1,3,8]. In brief, a stainless-steel cannula (outer diameter, 0.35 mm) inclined at 20° from the vertical line, 2 mm posterior, 3.4 mm lateral to the bregma, 8.5 mm deep from the surface of the cortex) was implanted in the third cerebral ventricle for continuous icv infusion. Three cortical gold-plated screw electrodes and paired stainless-steel electrodes were fixed to the skull with dental acrylic resin for recording the EEG and EMG, respectively; the cable from the electrodes was attached to a socket. After implantation of the cannula and attachment of the electrodes, animals were administered penicillin G potassium (20,000 IU, subcutaneously) and were allowed to recover for 10 days.

Following the recovery period, animals were transferred to individual experimental cages that allowed continuous icv infusion and monitoring of EEG and EMG. The experimental cages were placed in a soundproof, electromagnetically shielded room with the same environmental conditions as described above. Polyethylene tubing (PE10, 0.28 mm, inner diameter) was used to connect the cannula to an infusion pump (ESP-32, Eicom, Kyoto, Japan) set for continuous infusion of normal saline with or without the experimental peptides at a rate of 10 μl/h. During the 7 days preceding the experiment, animals underwent daily continuous infusion of normal saline in the experimental chamber to acclimatize the animals to the infusion and recording conditions.

During measurement, the electrode leads were connected to an EEG/EMG amplifier (MEG-6116, Nihon-Kohden, Tokyo, Japan) via a 5-strand cable with a slip-ring that enabled the rats to move freely. The amplifier was connected to a personal computer with an AD converter and software (SleepSign, Kissei Comtec, Nagano, Japan) for acquiring and processing data. Data were sampled at 128 Hz and subjected to online spectral analysis by Fast Fourier Transformation over 8-s epochs. Data were stored on a magnetic optical disk and subsequently analyzed visually offline after auto-analysis of the sleep–wake stages. EEG and EMG were monitored for three consecutive days [(Day 1, Baseline; Day 2, Experiment (orexin-B or α-FMH + orexin-B); Day 3, Recovery)] during continuous icv saline infusion at a rate of 10 μl/h. For a 5-h diurnal period, orexin-B (10 nmol/50 μl saline) replaced the icv infusion of saline. α-FMH (100 mg/kg, i.p.) [6,11] was administered 6 h before icv infusion of orexin-B [19].

The sleep–wake state was classified as wakefulness (W), REM sleep, and non-REM sleep from the EEG and EMG recordings, and the results were verified visually according to the standard criteria [3,8]: wakefulness (high EMG amplitude, low EEG amplitude); non-REM sleep (low EMG amplitude, high EEG amplitude with high power density in the delta band (0.5–4.0 Hz)); and REM sleep (silent low EMG amplitude, low EEG amplitude with high values in the theta band (4.0–8.0 Hz)). The analyzed sleep variables included the amount of non-REM and REM sleep, and the number and duration of each sleep parameter episode. The durations of wakefulness, non-REM sleep, and REM sleep were determined for each hour of the 24-h recording.

2.4. Statistical analysis

Statistical analyses were performed using repeated measures analyses of variance (ANOVA) followed by post hoc analysis of significance using the Student–Newman–Keuls test. Probability (p)-values less than 0.05 were considered to indicate statistical significance.

3. Results

Sleep–wake activity was evaluated and EEG data were analyzed at baseline (vehicle-treated) during the icv administration of orexin-B and during pretreatment with α-FMH i.p. 6 h before the

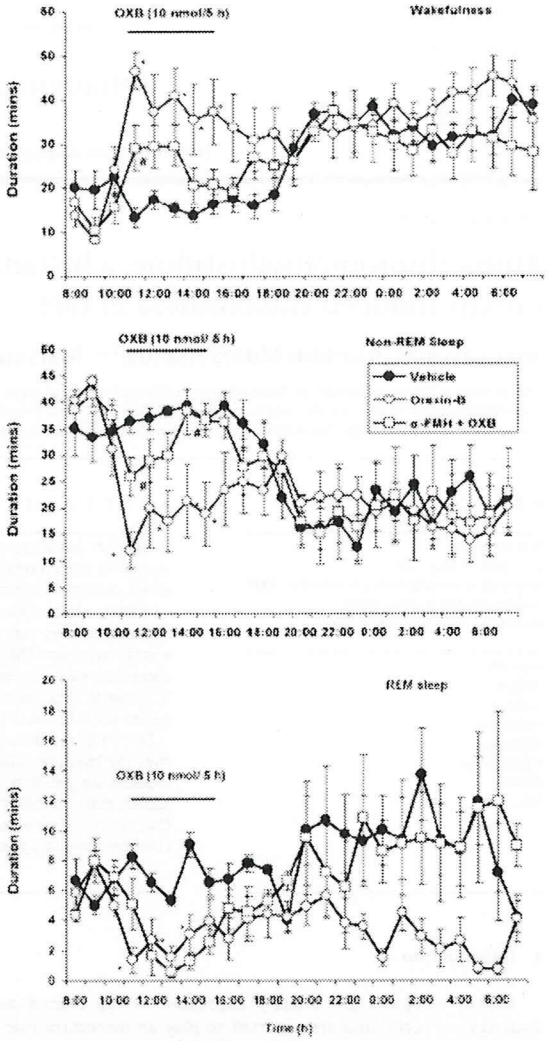

Fig. 1. Effects of vehicle (saline), orexin-B (10 nmol), and α-FMH + OXB on the time-course of wakefulness, non-rapid eye movement (non-REM) sleep and rapid eye movement (REM) sleep in rats. *$p < 0.05$; compared with vehicle-treated rats and #$p < 0.01$ compared with orexin-B. Vehicle, baseline; OXB, orexin-B; α-FMH, alpha-fluoromethyhistidine. Values are presented as mean ± SEM [vehicle, $n = 12$; orexin-B, $n = 6$; and α-FMH + OXB, $n = 6$]. The horizontal black bar denotes the onset and duration of icv infusion of orexin-B.

administration of orexin-B. The effects on sleep–wake stages are as shown in Fig. 1 for wakefulness, non-REM sleep and REM sleep on the time-course study with continuous icv saline (vehicle) infusion at a rate of 10 μl/h administration, orexin-B (10 nmol/50 μl saline), and pretreatment with α-FMH (100 mg/kg, i.p.) administered 6 h before icv infusion of orexin-B. The results show that orexin-B significantly ($p < 0.05$) increased wakefulness during the infusion period and that α-FMH significantly ($p < 0.05$) blocked this effect of orexin. Regarding non-REM sleep, the results showed that orexin significantly decreased this sleep stage and that this orexin-B effect was blocked with prior administration of α-FMH. Fig. 2 presents further analysis regarding the total time spent during the 5-h infusion of orexin-B (10 nmol) and the influence of α-FMH on the effect of orexin-B for wakefulness [$F(2,23) = 213.25$, $p = 0.0001$], non-REM

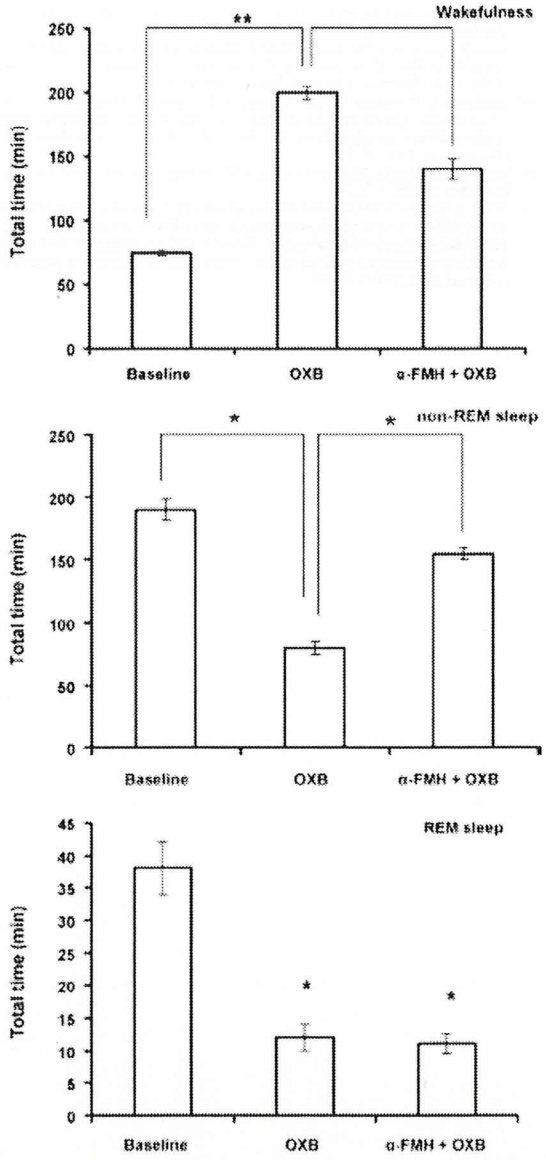

Fig. 2. Total time spent in wakefulness, non-rapid eye movement (non-REM) sleep and rapid eye movement (REM) sleep during the 5-h infusion of orexin-B (10 nmol) into the third ventricle of rats and intraperitoneal administration of α-FMH prior to administration of orexin-B (α-FMH + OXB). The columns represent baseline (vehicle) and experimental day profiles. Values are presented as mean ± SEM (baseline, $n = 12$; orexin-B, $n = 6$; and α-FMH + OXB, $n = 6$). $^*p < 0.05$.

sleep [$F(2,23) = 51.10$, $p = 0.0001$], and REM sleep [$F(2,23) = 19.25$, $p = 0.0001$], respectively.

4. Discussion

Orexin-B has affinity for OX_2R; a direct synaptic connectivity has been reported between orexin nerve terminals and histaminergic neurons in the TMN where OX_2R is abundantly expressed. Previously, we reported that icv infusion of both orexins A and B induced a significant arousal effect in rats where we established that orexin-B (orexin receptor type 2) plays significant role in the modulation of sleep wake stages [2]. In the present study, we established the involvement of the histaminergic system in the arousal effects of orexin-B by investigating the effects of intraperitoneal injection of α-FMH, a histamine synthesis inhibitor (histidine decarboxylase (HDC) suicidal), 6 h prior to icv infusion of orexin-B in rats. The results showed that continuous infusion of orexin-B at a dose of 10 nmol/50 μl/saline has a significant effect on sleep–wake stages: it significantly increased wakefulness, with a concomitant decrease in both NREM sleep and REM sleep.

A previous study reported that the extracellular histamine level in the frontal cortex is positively correlated with the amount of wakefulness in rats, thus indicating that histamine release in the cortex is strongly related to the sleep–wake cycle [5]. Furthermore, i.p. administration of α-FMH has been reported to significantly decrease the brain histamine content in the hypothalamus, cortex, and thalamus, and significantly affect locomotor activity [16,17]. Alpha-FMH is reported to potentiate thiopental-induced sleep in both mice and rats [12]. Thus, because orexin neurons are reported to densely innervate TMN neurons where OX2R is abundantly expressed, and considering the results of previous studies, this may suggest that orexin-B neurons are involved in the regulation of arousal state via the modulation of histaminergic neurons in the TMN. In the present study, we observed that prior treatment with α-FMH antagonized the sleep–wake modulating effect of orexin-B. The results revealed that α-FMH significantly reversed the increase in wakefulness and caused a significant differential effect on both non-REM and REM sleep with a significant prevention of decrease in hourly effects on non-REM sleep induced following administration of orexin-B, which has been known to act on orexin-2-receptors in the brain without any significant effect on REM sleep.

In conclusion, in the present study we established the involvement of the histaminergic system in the arousal-induced effects of orexin-B in rats, which further supports the hypothesis that the sleep–wake effect of orexin-B strongly depends on the activation of histaminergic neurotransmission.

Acknowledgement

This study was supported by a Grant-in-Aid for Scientific Research (C) (No. 14570912, 16614003) to K.H.

References

[1] Akanmu MA, Honda K, Inoue S. Hypnotic effects of total aqueous extracts of *Vervain hastata* (Verbenaceae) in rats. Psych Clin Neurosci 2002;56:309–10.
[2] Akanmu MA, Honda K. Selective stimulation of orexin receptor type 2 promotes wakefulness in freely behaving rats. Brain Res 2005;1048:138–45.
[3] Akanmu MA, Songkram C, Kagechika H, Honda K. A novel melatonin derivative modulates sleep-wake cycle in rats. Neurosci Lett 2004;364:199–202.
[4] Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999;98:437–51.
[5] Chu M, Huang ZL, Qu WM, Eguchi N, Yao MH, Urade Y. Extracellular histamine level in the frontal cortex is positively correlated with the amount of wakefulness in rats. Neurosci Res 2004;49:417–20.
[6] Fernández-Novoa L, Franco-MAside A, Alvarez XA, Cacabelos R. Effects of histamine and alpha-fluoro methylhistidine on brain tumor necrosis factor levels in rats. Inflamm Res 1995;44(1):55–7.
[7] Gerashchenko D, Kohls MD, Greco MA, Waleh NS, Salin-Pascual R, Kilduff TS, et al. Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behaviour in the rat. J Neurosci 2001;21:7273–83.
[8] Honda K, Komoda Y, Inoué S. Oxidized glutathione regulates physiological sleep in unrestrained rats. Brain Res 1994;636:253–8.
[9] Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999;98:365–76.
[10] Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 2001;435:6–25.

[11] Monti JM, D'Angelo L, Jantos H, Pazos S. Effects of fluoromethylhistidine on sleep and wakefulness in the rat. J Neural Transm 1988;72(2):141–5.
[12] Otshi R, Itoh Y, Nishibori M, Saeki K, Ueki S. Enhancement by alpha-fluoro methylhistidine of the thiopental sleep-prolonging action of delta 9-tetrahydrocannabinol. Psychopharmacology (Berl) 1988;95(1):77–81.
[13] Onodera K, Yamatodani A, Watanabe T. Effects of alpha-fluoro methylhistidine on locomotor activity, brain histamine and catecholamine rats. Methods Find Exp Clin Pharmacol 1992;14(2):97–105.
[14] Peyron C, Tighe DK, Van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998;18:9996–10015.
[15] Ramesh V, Thakkhar MM, Strecker RE, Basheer R, McCarley RW. Wakefulness-inducing effects of histamine in the basal forebrain of freely moving rats. Behav Brain Res 2004;152:271–8.
[16] Sakai N, Sakurai E, Onodera K, Asada H, Miura Y, Watanabe T. Long-term depletion of brain histamine induced by alpha-fluoro methylhistidine increases feeding-associated locomotor activity in mice with a modulation of brain amino acid levels. Behav Brain Res 1995;72(1–2):83–8.
[17] Sakai N, Yamazaki S, Onodera K, Yanai K, Maeyama K, Watanabe T. Effects of (S)-alpha-fluoro methylhistidine and (R)-alpha-methylhistamine on locomotion of W/Wv mice. Pharmacol Biochem Behav 1993;46(1):95–9.
[18] Sugimoto K, Maeyama K, Alam K, Sakurai E, Onoue H, Kasugai T, et al. Brain histaminergic system in mast cell-deficient (Ws/Ws) rats: histamine content, histidine decarboxylase activity, and effects of (S)alpha-fluoromethylhistidine. J Neurochem 1995;65:791–7.
[19] Sweet DC, Levine AS, Billington CJ, Kotz CM. Feeding response to central orexins. Brain Res 1999;821:535–8.
[20] Willie JT, Chemelli RM, Sinton CM, Yanagisawa M. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 2001;2:429–58.
[21] Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan JL, Wang QP, et al. Orexins activate histaminergic neurons via the orexin 2 receptor. Biochem Biophys Res Commun 2002;290:1237–45.