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REVIEW ARTICLE

Paradoxical action of zolpidem: interplay between dysregulation of the synergetic actions of γ-aminobutyric acid type A receptors and neuronal cotransporters (KCC2/NKCC1)

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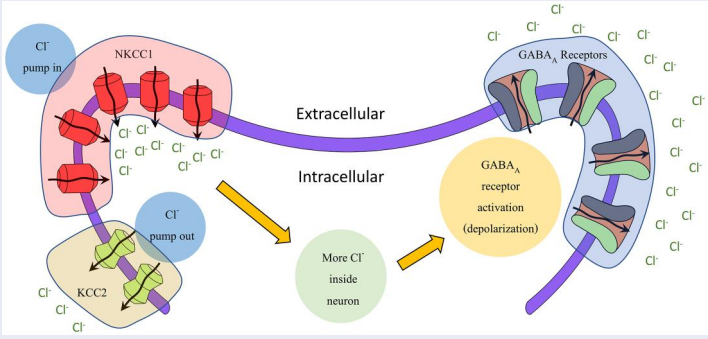
ABSTRACT

Zolpidem, or commercially known as Ambien or Stilnox, is a sedative–hypnotic agent, which is usually prescribed to manage sleeping difficulties in individuals with insomnia. The site of its sedative–hypnotic action is the γ-aminobutyric acid type A receptor, which it shares with benzodiazepines. However, this substance has been consistently associated to awaken patients with brain injuries such as trauma, stroke, disorders of consciousness and has also been expanded to the recovery of brain function in the event of hypoxic damage, cerebrovascular ischemic injury, infection of the central nervous system, toxins and poisoning, degenerative diseases, tumors, and congenital disorders. Aside from that, the effect has been observed in a wide spectrum of neurological diseases, from movement disorders such as Parkinson's disease and dystonia to neurological deficits and anaphylactic hypoxia. The wide spectrum of injuries and disorders reported poses a challenge in investigating the exact mechanisms of action underlying the awakening effect. Therefore, the main question is how it is possible for a substance originally intended as a sedative–hypnotic agent to induce an awakening effect? In this review, we discuss the synergetic roles of $GABA_A$ receptors, which are the target receptor for zolpidem, along with neuronal cation-Cl^- co-transporters KCC2/NKCC1 in regulating the inhibitory nature of the GABAergic transmission in brain injury, which might explain the awakening effect of zolpidem in brain injuries.

GRAPHICAL ABSTRACT



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Introduction

Zolpidem: from sedative–hypnotic to awakening agent

Zolpidem ($C_{19}H_{21}N_3O$) or systematic name N,N,6-trimethyl-2[4-methylphenyl]imidazo[1,2-a]pyridine-3-acetamide hemitartrate (Figure 1), belongs to the class of imidazopyridine compounds and was first synthesized in France by Syntelábo Recherche in the 1980s [1]. Commercially known as Ambien or Stilnox, this so-called Z-drug is a sedative–hypnotic agent, which is usually prescribed in a dose of 5–10 mg to manage sleeping difficulties in individuals with insomnia [2]. The pharmacological formulation is provided as zolpidem tartrate, an off-white crystalline powder that is soluble in water, alcohol, and propylene glycol [3]. In terms of pharmacokinetics, zolpidem achieves peak plasma concentration between 0.75 to 2.6 h, and its elimination half-life ranges from 1.5 to 3.2 h [4]. The site of its

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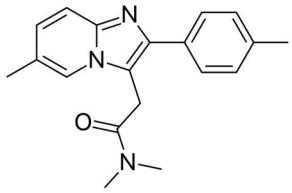

Figure 1. Depiction of zolpidem chemical structure.

sedative–hypnotic action is the γ-aminobutyric acid type A receptor ($GABA_AR$), which it shares with benzodiazepines [5]. However, zolpidem appears to exhibit minimal anxiolytic, myorelaxant, and anticonvulsant side-effects that are commonly associated with benzodiazepine site agonists [6]. Therefore, zolpidem is fast becoming the preferred drug over benzodiazepines for inducing sleep in patients with sleep disorders [3].

Interestingly, contradictory to its actual action as a sedative-hypnotic, zolpidem has been consistently associated to awaken patients with brain injuries such as trauma, stroke, and disorders of consciousness (DOC). This indication has also been expanded to the recovery of brain function in the event of hypoxic damage, cerebrovascular ischemic injury, infection of the central nervous system (CNS), toxins as well as poisoning, degenerative diseases, tumors, and congenital conditions [7,8]. Furthermore, this awakening effect has been observed in a wide spectrum of neurological diseases, from movement disorders such as Parkinson's disease (PD) and dystonia to neurological deficits and anaphylactic hypoxia [9–11]. Intriguingly, the effect has also been replicated and reported by numerous researchers in different brain injury studies [10–17]. Indisputably, the wide spectrum of injuries and disorders reported poses a challenge in investigating the exact mechanisms of action underlying the awakening effect. Therefore, the use of zolpidem for the treatment of non-insomnia disorders, such as brain injury, has not found a foothold in mainstream clinical practice.

Considering that the modulatory action of zolpidem is exerted through the inhibition of neurotransmission, mediated by $GABA_ARs$, its awakening effect is sometimes referred as the paradoxical action of the drug, as it reflects an effect that is opposite to its intended action. Given its paradoxical potential in aiding recovery from neurological disorders, studies investigating the mechanism of action of zolpidem may offer substantial scientific evidence and have profound clinical value. However, due to lack of pre-clinical investigations, insufficient scientific evidence on the cellular and physiological mechanisms underlying this paradoxical action and wide range of responses to zolpidem remain unelucidated. This review focuses on the paradoxical actions of zolpidem, emphasizing the complex interplay between $GABA_ARs$ and the neuronal chloride cotransporters KCC2 and NKCC1. It synthesizes current evidence suggesting that dysregulation of these cotransporters impairs chloride homeostasis and alters GABAergic signaling, potentially shifting zolpidem's action from inhibitory to excitatory in pathological brain states. By evaluating potential molecular mechanisms and clinical observations, this review aims to clarify the factors contributing to zolpidem's divergent effects and to identify future research directions that could enhance therapeutic applications for brain injury and related neurological disorders.

An awakening effect of zolpidem in selected cases

Zolpidem was first reported to exhibit the paradoxical arousal effect in patients with brain injury in 2000 by a study that described a male patient with intra-cerebral hemorrhage in the left lentiform nucleus and thalamic area caused by a vehicular accident [18]. The patient's mental status was a semi-coma and he also exhibited deficits in muscle control (poor sphincter control) in addition to impaired speech comprehension (no verbal response to commands and questions). After several months, the patient became intensely restless. Therefore, zolpidem (10 mg) was prescribed to calm him. Unexpectedly, 15 min after zolpidem ingestion, the patient appeared to transiently regain consciousness and was able to greet his mother verbally with the words 'Hello, Mom' [16]. Following this remarkable improvement, zolpidem was administered in the morning, during which the patient's consciousness level seemed to be alert but relapsed to the semi-comatose state in the afternoon and night. During the conscious phase, he was able to verbally respond to simple questions and exhibited intact cognitive and memory function. The signs of disturbance of physical sensation and muscle control such as hyperesthesia, spasticity, and chorea were markedly decreased during the conscious phase. Thereafter, Clauss and colleagues have extensively studied the effect of zolpidem on cerebral perfusion flow using single-photon emission computed tomography (SPECT) in primate models [19,20] as well as humans [18,21].

According to another clinical report, zolpidem (10 mg) administration to three patients suffering from brain injury (two were caused by vehicular accidents, whereas the third was due to near-drowning) resulted in an arousal effect in all three patients [22]. Before being prescribed zolpidem, the patients reportedly showed impaired muscle coordination, difficulty understanding speech, and visual and auditory disturbances. Zolpidem significantly improved the Glasgow Coma Scale score in all patients, and the arousal effect lasted for approximately 4 h. Another study by [23] that used positron emission tomography (PET) to evaluate the change in the regional brain metabolism after zolpidem 10 mg administration in three patients with a post-anoxic minimally conscious state (MCS) also yielded consistent results. The patients showed improvement in auditory, visual, motor, and verbal functions after zolpidem administration. Additionally, the findings of PET indicated that all patients who were given zolpidem exhibited increased brain metabolism in specific brain areas including the frontal (e.g. Brodmann area 8, 9, 10 and 47) and parietal lobes (Brodmann area 40), reflecting an enhanced metabolism effect induced by zolpidem in the post-anoxic MCS brain. Other PET studies also corroborated similar patterns of increased cerebral perfusion flow in the anterior cingulate gyrus, orbitofrontal cortices, striatum, and thalamus in patients with post-anoxic encephalopathy after zolpidem administration [12,24].

The paradoxical effect of zolpidem in a 48-year-old male patient suffering from brain injury due to myocardial infarction was reported in 2016 [25]. Due to brain injury, the patient exhibited disturbances in consciousness (the patient was in semi-comatose phase), and motor and speech function, in addition to agitated behavior. The patient was administered zolpidem (10 mg) to ameliorate agitation. Consistent with the previously reported series of clinical cases, the patient exhibited improved motor function and communication, which lasted for 2–3 h after zolpidem administration [18,22]. Additionally, in a recent study, a woman was admitted to the emergency room due to attempted suicide [26]. She arrived in a comatose state and experiencing cardiac arrest. Emergency procedures were implemented including resuscitation of the cardiopulmonary and cerebral function to recover spontaneous circulation. Throughout the post-operative hospitalization period, she exhibited drowsiness, movement disturbance including dysphagia and quadriparesis, impaired cognitive function, and sleep disturbance. Zolpidem (5 mg) was administered as hypnotic therapy for an insomniac episode. Paradoxically, the patient regained a decent level of consciousness, improved verbal comprehension, and motor function (e.g. absence of dysphagia and quadriparesis). Due to this paradoxical effect, the patient was empirically administered zolpidem twice a day. She exhibited and performed near-normal activities, including exercises and normal communication with other people throughout the treatment regimen.

Several clinical trials investigated the effect of zolpidem in patients with brain injury using SPECT and revealed the correlation between the SPECT findings and clinical improvements [11]. A clinical study employed a similar method where 127 patients with unresponsive wakefulness syndrome (formerly known as a vegetative state) following brain injury received a daily dose of zolpidem 10 mg for one week and underwent SPECT imaging [13]. It concluded that zolpidem can restore brain function in these patients, especially when the injury did not affect the brain stem region. Electroencephalography was used to compare brain activity of patients who showed to response to zolpidem before and after administration of the drug [27]. Interestingly, before zolpidem administration, these patients exhibited more synchronized and lower frequency brain activity compared to healthy participants; however, after zolpidem administration, the synchronicity decreased, and the frequency of the brain waves elicited was higher. These results represent the firing activity of hippocampal and cortical neurons during wakefulness [28]. Furthermore, zolpidem improves motor function in patients with generalized dystonia, Meige syndrome, hand dystonia, dementia, spino-cerebellar ataxia, PD, and progressive supranuclear palsy [10,16].

The Australian Broadcasting Corporation (ABC) channel aired Sam Goddard's story in 2017 (I am Sam – Australian Story), featuring a documentary about a patient who experienced a series of strokes that left him with unresponsive wakefulness syndrome. At a relatively young age of 23 years, the physician did not hope for improvement in his condition, but his fiancée convinced the doctors to administer zolpidem to him [29]. Shortly, within a few days, the awakening effect of zolpidem was observed when Sam managed to communicate at a comprehensible, although subnormal level, before the effects gradually disappeared when the drug left his system and improved again upon re-administration of zolpidem. However, other clinical case studies have reported differing results, noting that the occurrence of zolpidem-induced paradoxical consciousness in patients with brain injury was uncommon and mild; only 4.86.7% of patients with brain injury showed clinically significant responses to zolpidem [17,30]. The paradoxical effect was not reproducible in a study conducted by [8], where all the participants failed to exhibit significant clinical recovery following zolpidem treatment. In addition to its inconsistent clinical reproducibility, the (time to) onset of action has been shown to diminish after chronic (18 months to 3 years) zolpidem administration [26].

Essentially, clinical bedside behavioral assessment (e.g. clinical assessment for recovery of consciousness, cognitive, verbal, auditory and motor functions) alone may not be sufficient to explain the discrepancy in the recovery pattern and decreased drug effectiveness in patients experiencing this perplexing paradoxical reaction. The discrepancies in findings also hinder attempts aimed at understanding the effect mediated by zolpidem in brain injury, engendering a new perspective that the nature of injury may contribute to this paradoxical action. Moreover, the scarcity of pre-clinical animal studies on the paradoxical action of zolpidem signifies the need for further investigations to clarify this ambiguity and build a body of robust scientific evidence.

The rarity of the awakening effect and its irreproducibility hamper detailed scientific elucidation. Therefore, research employing diverse experimental models, both *in vitro* and *in vivo*, is necessary to clarify the paradoxical effects of zolpidem in brain injury and to gain insights into its potential neuroprotective and neurotherapeutic effect in patients with brain injury, which may provide basic evidence for clinical neurology and neuroscience studies. Therefore, the research question is very obvious – how is it possible for a substance originally intended as a sedative–hypnotic agent to induce an awakening effect? Unequivocally, this process involves $GABA_AR$, a major inhibitory neurotransmitter receptor since it is the target receptor for zolpidem.

$GABA_A$ receptors: potential targets for the awakening action of zolpidem

$GABA_ARs$ serve as the primary inhibitory receptors in the CNS. They belong to the ligand-gated ion channel superfamily, along with nicotinic acetylcholine (nACh), 5-hydrotryptamine type-3 ($5HT_3$), and glycine receptors (GlyRs) [31]. In the CNS, $GABA_ARs$ are activated by GABA, the main inhibitory neurotransmitter [32]. *via* $GABA_ARs$, GABA mediates various neuronal developmental processes including synaptic formation as well as neuronal migration, growth, and proliferation [33]. GABAergic neurons are defined as neurons that synthesize and release the GABA neurotransmitter. GABA released into the synaptic cleft binds to post-synaptic receptors. $GABA_ARs$

are pentameric transmembrane protein complexes composed of various combinations of 19 identified subunits: 6 α (α1–6), 3 β (β1–3), 3 γ (γ1–3), as well as δ, ε, θ, π, and 3 ρ (ρ1–3), which give rise to different receptor isoforms [34,35]. Regarding the receptor subunit composition or subtype, α1β2γ2 is the most prevalent $GABA_AR$ subtype in the CNS, characterized by a 2:2:1 stoichiometry – comprising two α1, two β2, and one γ2 subunits—arranged in an anti-clockwise sequence of γ2β2α1β2α1 [36], whereas minor subunits such as γ are usually replaced by δ or ε in the extra-synaptic $GABA_ARs$ [37]. This subunit complex pseudo-symmetrically encompasses a central pore channel in the γ-β-α-β-α sequence in an anti-clockwise fashion when viewed from the extracellular side [38,39]. Through $GABA_ARs$, zolpidem exerts its action on the same site as benzodiazepines, i.e. the α(1–3)/γ2 subunit interface, and positively modulates receptor function by increasing the receptor's channel opening frequency, thereby enhancing Cl^- influx [5,40,41]. This influx of Cl^- leads to neuronal hyperpolarization, which in turn inhibits neuronal excitability and suppresses neurotransmission, contributing to zolpidem's sedative–hypnotic effects. However, in our recently published study using a status epilepticus (SE) animal model, we observed that the sedative–hypnotic effects of zolpidem were markedly diminished, suggesting a disruption or alteration of $GABA_AR$-mediated inhibitory signaling in this pathological condition [42].

Zolpidem exhibits high affinity for α1-containing $GABA_ARs$ and intermediate affinity for α2- and α3-containing $GABA_ARs$ [43,44]. Several drug and substance binding sites on $GABA_ARs$ are situated at the interfaces between adjacent receptor subunits. Thus, the position or arrangement of these subunits plays a critical role in forming the structure of the binding site, which eventually determines the pharmacological properties of the receptor. Rationally, the same drug could exert varying pharmacological effects if it binds to a different site. Previous studies have indicated that the arrangement depends on the expression levels of the subunits, which can be potentially altered in brain injury and trauma, in addition to other situations such as neurological disorders. Coincidentally, several studies have reported the effects of injury on brain receptors, including $GABA_ARs$. Ischemic insult can influence the protein expression of the α1 subunit [45], which is crucial for zolpidem binding. Stroke can also decrease the mRNA expression levels of β subunits, which are responsible for both GABA binding sites in $GABA_ARs$ [46]. Meanwhile, detailed studies have shown the effect of induced traumatic brain injury (TBI) on $GABA_AR$ subunits such as α1, α2, α5, β2, β3, γ2, and δ in animal models [47]. Altered expression and rearrangement of a single subunit of $GABA_ARs$ could have a profound implication for the subtype and, more importantly on the binding sites of the receptors. This observation is supported by a study on the association of the ε subunit with temporal lobe epilepsy [48]. It was demonstrated that the benzodiazepine sensitivity of synaptic receptors in hippocampal dentate granule cells disappeared following TBI induction, suggesting interruption at the binding site of these drugs, which is most likely caused by alteration in subunit expression [49].

The existence of a potential alternative binding site (or sites) for zolpidem on $GABA_ARs$ has been proven by *in vitro* studies. One such study conducted in 2016 [50] investigated the effects of subunit arrangement of $GABA_ARs$ by altering the expression ratio of each subunit using two-electrode voltage-clamp electrophysiology. Binary $GABA_A$ α1β3 receptors expressed in 3α1:2β3 stoichiometry were modulated by zolpidem, whose effect was attenuated by flumazenil. The α1-α1 subunit interface of the 3α1:2β3 $GABA_AR$ contains a zolpidem-binding site, akin to the classical α1-γ2 interface. Although this arrangement is yet to be discovered in *in vivo* samples, this *in vitro* study proved that altering the expression of each subunit could create functional $GABA_ARs$. Evidently, this finding supports the hypothesis that rearrangement of each subunit within $GABA_AR$ subtypes is possible and could create different binding sites for zolpidem. Therefore, it is believed that brain injury could have affected the expression and arrangements of the $GABA_AR$ subunits. However, the mechanism by which these alterations build potential binding sites remains undetermined and could explain the intriguing effects of zolpidem through the binding process at the site.

Physiologically, the inhibitory effect of $GABA_ARs$ is mainly rendered by neuronal Cl^- homeostasis, and Cl^- current directionality and magnitude. In the physiological state, binding of GABA at the β/α subunit interface of $GABA_ARs$ results in the net influx of Cl^-. In the presence of a positive modulator, it potentiates the net influx of Cl^-, enhancing the inhibitory GABA-mediated Cl^- current. The influx of Cl^- along the electrochemical gradient in physiological states is mainly due to the difference in the electrochemical gradient of Cl^- between the intra- and extracellular regions, i.e. lower intracellular Cl^- concentration, $[Cl^-]_i$, relative to the extracellular Cl^- concentration, $[Cl^-]_o$. This phenomenon is primarily established and maintained by the action of neuronal cation-Cl^- co-transporter (CCC) proteins. Moreover, physiological Cl^- homeostasis, which is maintained by CCCs, significantly underpins the inhibitory nature of the GABAergic transmission in the CNS. Furthermore, $[Cl^-]$i dictates the polarity and intensity of GABAergic transmission mediated by $GABA_ARs$ in the nervous system [51].

Neuronal cation-Cl^- co-transporters

K^+-Cl^- cotransporter (KCC) 2 and Na^+–K^+–Cl^- cotransporter (NKCC) 1 are the primary cotransporters regulating intracellular Cl^- homeostasis, which is critical for the GABAergic inhibitory effect. The solute carrier 12 (SLC12) family of the CCCs is composed of four KCCs (KCC1 encoded by *SLC12A4*, KCC2 encoded by *SLC12A5*, KCC3 encoded by *SLC12A6,* and KCC4 encoded by *SLC12A7*), two NKCCs (NKCC1 encoded by *SLC12A2,* and NKCC2 encoded by *SLC12A1*) and one Na^+-Cl^- cotransporter (NCC encoded by *SLC12A3*) [52]. The KCC and NKCC families comprise distinct proteins that are specifically expressed throughout different organs and cell types [52,53]. KCC4 is expressed in the kidney, liver, lung, heart, and gastric luminal membrane [54,55]. KCC3 has two variants, viz. KCC3a and KCC3b. KCC3a is expressed in the heart, lung,

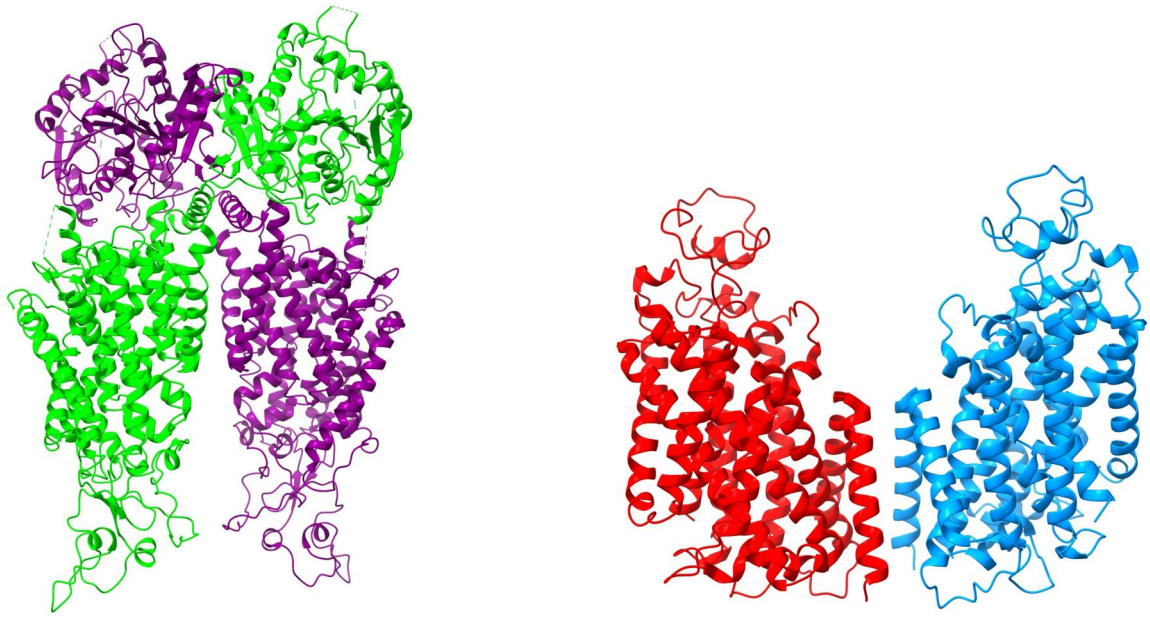

Figure 2. Ribbon illustration of KCC2 dimeric structure comprising of two subunits (purple and green – left) and NKCC1 dimeric structure comprising of two subunits (red and blue – right). Figure adapted from PDB ID 6M23 for KCC2 (94) and 6PZT for NKCC1 [95].

muscle, and brain, whereas KCC3b is found predominantly in the kidney [55]. KCC2 is specifically expressed in neurons of the CNS, such as in the hippocampus, cerebellum, cortex, and brain stem [56,57]. KCC2 is also specific to neurons and absent from glial cells [58]. KCC2 is vastly expressed in the soma and dendrites of pyramidal neurons in the hippocampus and neocortex [59]. KCC2 exhibits 67% amino acid sequence similarity to KCC1 in the rat brain and rabbit kidney [56].

NCC and NKCC2 are not expressed in the brain and are predominantly expressed in the kidney, especially in the renal medulla and cortical ascending limb [52,59,60]. NKCC1 is expressed in the peripheral and central neurons, including glial cells [59,61,62]. NKCC1 has two splice variants, i.e. NKCC1a and NKCC1b, and the former is highly expressed in the brain [59,63]. KCC2 and NKCC1 possess similar secondary protein structures composed of twelve transmembrane protein domains, six extracellular loops, and intracellular N- and C-terminals [62,64,65]. KCC2 forms monomeric and dimeric structures but not trimeric or higher oligomeric structures (Figure 2) [64,66]. Meanwhile, NKCC1 forms dimeric structures in order to be functional, despite having potential monomeric expression (Figure 1) [62,65,66,67]. Both proteins exhibit different phosphorylation sites: the most studied KCC2 phosphorylation sites are Ser940, Thr1007 and Ser1022 [64], whereas Thr197, Thr201, Thr206, Thr211 and Thr224 are the most studies phosphorylation loci for NKCC1 [62].

Synergetic roles of KCC2 and NKCC1 in the regulation of intracellular Cl^-

A steady intracellular Cl^- concentration gradient is crucial for maintaining neuronal function and is primarily regulated by two cell-type-specific co-transporters: KCC2 and NKCC1 [59]. KCC2 mediates the efflux of K^+ and Cl^-, promoting low intracellular Cl^- levels in mature neurons, whereas NKCC1 drives Na^+, K^+, and Cl^- influx, typically active in immature or pathologically altered neurons [59]. This intracellular Cl^- regulation supports vital neuronal processes including growth, cell volume control, pH regulation, and the establishment of the Cl^- equilibrium potential [68]. In mature neurons, high KCC2 expression and low NKCC1 activity establish a steep Cl^- gradient, resulting in intracellular concentrations around ~5 mM versus ~110 mM extracellularly [32,69]. This gradient ensures that $GABA_AR$ activation leads to Cl^- influx and neuronal hyperpolarisation, producing an inhibitory effect [52,68]. Conversely, in immature or pathological states – where NKCC1 is upregulated and KCC2 downregulated – this gradient is reversed, leading to Cl^- efflux and GABA-induced depolarization and excitation [59,70]. This dysregulation not only alters physiological function but also impairs the efficacy of GABAergic drugs. This disrupted chloride homeostasis has direct implications for the pharmacological action of $GABA_AR$ modulators such as antiepileptic drugs (AEDs) and zolpidem. Under normal conditions, zolpidem enhances $GABA_AR$-mediated Cl^- influx, promoting neuronal inhibition and sedation. However, in pathologically altered neurons with reversed Cl^- gradients, zolpidem may potentiate Cl^- efflux, leading to paradoxical neuronal excitation. This mechanism is proposed to underlie the 'awakening' effects observed in some brain-injured patients [33,71,72]. Therefore, altered Cl^- gradients may render traditional GABA-enhancing treatments less effective or even counterproductive. While the possibility of restoring KCC2 and NKCC1 expression holds therapeutic promise, its feasibility and impact on functional recovery in brain injury remain to be determined. Understanding the complex regulation of these cotransporters is thus critical for developing targeted therapies to normalize chloride homeostasis and improve clinical outcomes (Figure 3).

Further evidence from animal models supports the dysregulation of KCC2 and NKCC1 expression in various forms of

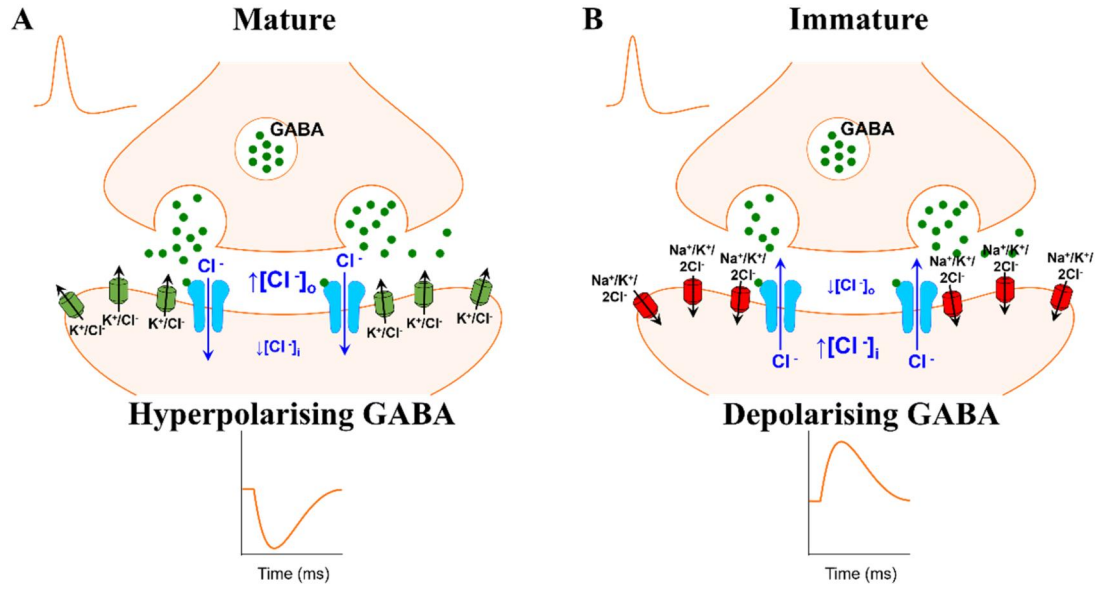

Figure 3. Illustration of KCC2 and NKCC1 expressions in mature and immature conditions. (A) In the mature neurons, KCC2 expression is highly expressed, which results in the mass extrusion of Cl^-. this event contributes to lower intracellular Cl^- concentration compared to the extracellular region. GABA action on $GABA_A$Rs results in the influx of Cl^- down its electrochemical gradient, hyperpolarising the post-synaptic neurons. (B) However, in an immature neuron, KCC2 expression is significantly lower in comparison to NKCC1. Due to KCC2 deficiency and NKCC1 excess, Cl^- is vastly intruded into the neurons resulting in the augmentation of intracellular Cl^- concentration, thereby shifting the electrochemical gradient of Cl^-. Concentration of the intracellular Cl^- is relatively higher than of the extracellular region. Therefore, GABA action on $GABA_A$Rs results in the outflux of Cl^-, thereby depolarizing the post-synaptic neurons. When zolpidem is present as a positive allosteric modulator, this inhibitory effect is enhanced due to increased $GABA_A$R channel opening frequency, further promoting Cl^- influx and neuronal hyperpolarization.

brain injury. For instance, studies have shown persistent downregulation of hippocampal KCC2 up to 45 days following SE induction in rats [73,74], indicating a long-term disruption of chloride extrusion. Similar reductions in KCC2 expression have been reported in models of transient focal cerebral ischemia [75], post-stroke spasticity [76], neuropathic pain [77], seizures [78], and peripheral nerve injury [79]. In contrast, NKCC1 expression is frequently elevated in these pathological conditions. A study by [74] observed sustained hippocampal NKCC1 overexpression 45 days post-SE, and similar trends have been reported in animal models of traumatic brain injury (TBI) [80], chronic epilepsy [81], and neonatal stress [82]. Consistent with these findings, we observed dysregulation of the cation-chloride cotransporters KCC2 and NKCC1 in our SE animal model [42]. This transporter imbalance may underlie the reduced efficacy of GABA-targeting agents like zolpidem and certain AEDs in brain-injured individuals. While the role of benzodiazepines under these conditions remains poorly characterized, it is increasingly evident that impaired chloride homeostasis – rather than direct receptor dysfunction – may be the primary factor in altered pharmacological responses. The potential reversibility of this dysregulation by zolpidem is of growing interest. Although clinical data are limited, a 2013 animal study found that chronic zolpidem administration in healthy mice led to upregulation of KCC2 in the limbic forebrain [83]. However, these findings may not directly translate to injured brains, where complex alterations in kinase signaling cascades – such as reductions in protein kinase C (PKC) isoforms [84,85], and elevations in STE20/SPS1-related proline–alanine-rich kinase (SPAK), with-no-lysine (K) or WNK, and Oxidative Stress-Responsive kinase 1 (OSR1) expression [86,87] – may affect the drug's impact. These molecular changes suggest that brain injury induces a dynamic and multifactorial disruption of chloride transporter regulation, which may critically influence zolpidem's therapeutic efficacy and paradoxical effects.

Studies have demonstrated downregulation of different PKC isoforms in SE-mediated injury animals [84,85], significant upregulation of SPAK mRNA and protein in the pilocarpine model of SE [86], and upregulation of WNK and OSR1 in patients with schizophrenia [69,87]. These findings may imply evident alterations in cascade proteins due to neuronal injury or disorders, which may underlie the discrepancies in the experimental findings. The restoration of KCC2 and NKCC1 expression may also serve to clarify the potential mechanisms underlying zolpidem tolerance in brain injury, wherein chronic administration reduces the drug efficacy and positive clinical results. This assumption may be confirmed by a recent case study reporting that patients with brain injury, who had previously been prescribed the drug, did not respond to zolpidem administration [88]. In light of the potential mechanisms mediated by zolpidem in restoring KCC2 and NKCC1 expression and/or functions, and also its indirect involvement in restoring Cl- dysregulation, the inconsistent and divergent clinical findings related to involvement of zolpidem in mediating paradoxical outcomes in brain injury should be addressed by future studies.

Mechanistically, the WNK–SPAK/OSR1 pathway is known to inhibit KCC2 *via* phosphorylation at a specific Thr6 residue [89]. GABAergic signaling further tunes KCC2 at the neuronal membrane through $GABA_A$R activity and Cl^--dependent phosphorylation of KCC2 Thr906 and Thr1007 residues [90,91]. The second messenger Cl^- controls the activity of WNK lysine deficient protein kinase 1 (WNK1), a serine-threonine kinase that senses Cl^- and regulates SPAK and

OSR1 [92]. Additionally, GABAergic activity rapidly modulates the surface diffusion and clustering of NKCC1 in mature hippocampal neurons by engaging WNK1 along with SPAK and OSR1, which directly phosphorylate NKCC1 at key Thr203, Thr207, and Thr212 residues [90]. Zolpidem might indirectly counteract these effects by reducing activation of this kinase cascade through GABAergic modulation. Simultaneously, PKC phosphorylates KCC2 at Ser940, stabilizing it at the membrane, while PP-1 promotes dephosphorylation and internalization [93]. In SE models, reduced PKC expression may impair this protective phosphorylation, but chronic zolpidem treatment might have potential to restore PKC levels and suppress PP-1 activity, promoting KCC2 retention at the neuronal surface. Collectively, these two pathways – reducing WNK–SPAK/OSR1-mediated inhibition and boosting PKC-driven stabilization – suggest that zolpidem may help reestablish KCC2 functionality and chloride homeostasis in damaged neurons. Clinically, this may also help explain emerging reports of zolpidem tolerance in brain-injured patients. In some cases, the paradoxical 'awakening' response appears to diminish over time with repeated use. This could reflect adaptive restoration of KCC2 and NKCC1 expression, reducing zolpidem's atypical excitatory effects. However, whether zolpidem directly modulates transporter expression in injured neurons remains unclear, and no definitive studies have yet confirmed its long-term efficacy or mechanism of action in human brain injury. Therefore, while preliminary evidence supports zolpidem's role in modulating chloride homeostasis, further research is urgently needed to determine whether targeting KCC2/NKCC1 regulation can be translated into consistent, effective treatment strategies for patients with brain injuries or chloride-related GABAergic dysfunction. Future investigations should focus on clarifying the temporal dynamics and molecular pathways of zolpidem's effects on chloride transporters in various brain injury models to guide clinical application.

Conclusion

The phenomenon of the awakening effect mediated by the sedative–hypnotic agent zolpidem in patients with neurological disorders, including DOC, brain injuries, and neurodegenerative diseases remains shrouded in mystery. Although this drug is widely prescribed as a 'sleeping pill', given its potential in aiding the recovery of brain function in neurological disorders, understanding the precise mechanism of action is of substantial scientific value, especially in the rehabilitation process. However, the scarcity of research hampers understanding of the precise nature of the pharmacological effects of zolpidem. Moreover, studies have not added to the existing data, limiting evidence and encumbering the ability to understand this drug in a wider context. Therefore, fundamental studies focusing on the role of zolpidem in the awakening effect should be conducted to acquire essential understanding of zolpidem as a therapeutic agent for brain injury by emphasizing the role of $GABA_A$Rs and neuronal cation cotransporters KCC2 and NKCC1. Subsequently, the interplay between brain injury, alteration in $GABA_A$Rs, binding profiles of zolpidem at alternative binding sites on the new receptor subtypes, and the dysregulation of Cl^- homeostasis through the roles of KCC2 and NKCC1 can be proven.

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Ethics statement

The are no animal and human subjects in this article and ethical approval is not applicable.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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