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Is the awakening produced by benzodiazepines due to excitatory actions of GABA?

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Abstract

Benzodiazepines (BZDs) such as Zolpidem can produce a temporary revival of patients who have been akinetic and apathic for years. The mechanisms underlying this "awakening" reaction are suggested globally to be related to an activation of gamma-aminobutyric acid (GABA) inhibitory systems. However, brain trauma or cerebro-vascular infarcts, like many other pathological insults, are associated with a shift of the polarity of GABA from inhibition to excitation consequently to an increase of intracellular chloride concentration ($[Cl^-]_i$) levels. Experimental and clinical observations suggest that BZDs generate paradoxical reactions in these conditions, hence the transient "awakening". The NKCC1 (Na-K-2Cl co-transporter isoform 1) chloride importer antagonist Bumetanide restores low $[Cl^-]_i$ levels and an efficient inhibitory drive. It is therefore suggested that the administration of Bumetanide might provide a persistent "awakening" by shifting GABAergic actions from excitation to inhibition and attenuating the mechanism underlying the apathic/akinetic state.

Introduction

Awakening, a temporary revival of patients who have been akinetic, apathic and with no reaction for years following a large brain damage, has been observed after administration of drugs even if the mechanisms underlying them remain unknown. Although rare, the awakenings produced briefly by "sleeping pills" are now well documented [1–3]. Arnst et al. recently reported spectacular effects of Benzodiazepines (BZDs) on a 29 years old patient after a severe hypoxic-ischemic brain injury and following a history of alcohol abuse [4]. The patient suffered from a severe impairment of arousal and difficulty to maintain an arousal state. Magnetic resonance imaging showed signs of diffuse atrophy without hydrocephalus. For 8 years the patient remained mute, akinetic, incontinent, had muscle rigidity and no affective reactions. Following a single dose of Zolpidem (10 mg), the patient "managed to walk while being supported by the staff and phoned his father, who had not heard his son's voice for years. Despite evident retrograde amnesia, going back three years before the brain injury, and an apparent hearing deficit, he was cheerful, alert, and showing interest in the people and objects surrounding him". These transient effects (2 h) were repeated only for 5 days with a progressive reduction of efficacy, and after that delay BZDs had no effects at all. The treatment could however be efficient for short periods of time subsequently on special occasions, provided that they are infrequent.

Electroencephalogram (EEG) and magnetoencephalogram source-spectral analysis indicate a small but significant increase of beta and gamma band after Zolpidem treatment. It is usually considered that Zolpidem restores globally the excitation/inhibition imbalance due to a reduced GABAergic inhibitory drive [1–5]. Williams and colleagues reported an abrupt reduction of 6-10 Hz oscillations and the coherence between the two hemispheres in 3 patients with known positive response to Zolpidem [2]. Unfortunately, the alterations produced in Zolpidem non-responders were not investigated. Similar

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observations have been made after severe ischemic brain damage and in patients with a variety of brain disorders including severe degenerative ones, notably Parkinson's disease [6–11]. These effects are interpreted as a general restoration of gamma-aminobutyric acid (GABA) inhibition that has been hampered by the insult. However, the exact underlying mechanism and the site of reduced GABAergic inhibitory drive remains conjectural as these cannot readily be determined from EEG measures. If GABAergic inhibition has been impacted, it is fundamental to identify whether and which element of GABAergic mechanisms is deficient to suggest a therapeutic avenue. Considering the wide range of actions of Zolpidem and other BZDs, it is not astonishing that their effects on awakening have not been identified.

Here, I suggest that the paradoxical effects of BZDs are due to a shift of the polarity of GABA actions triggered by the pathological insult: the excitatory actions of GABA in post ischemic networks are increased by BZDs leading to paradoxical reactions. Indeed, the polarity of GABA actions is determined by intracellular chloride concentration ($[Cl^-]_i$) levels. When they are elevated, GABA exerts depolarizing/excitatory actions; and when they are low, GABA exerts hyperpolarizing/inhibitory actions. The shift from hyperpolarizing/inhibitory to depolarizing/excitatory actions has been reported in a wide range of disorders including ischemic insults and degenerative disorders [12–15]. Experimental observations suggest that paradoxical actions of BZDs occur when neurons have high $[Cl^-]_i$ levels and excitatory actions of GABA [16–18]. Therefore, I suggest that the ephemerous effects of BZDs are due to high $[Cl^-]_i$ levels and consequent GABAergic excitation. In this scenario, brief awakening by BZDs calls for the combined use of BZDs and agents known to restore inhibition in order to transform the brief effect to a long lasting one.

Activity-dependent dynamic changes of GABAergic inhibition in disease

The degree of complexity of GABA actions is quite unsurpassed. Many parameters impact the efficacy of GABAergic signals and inhibition: the large number of subunits of GABA receptors that determine the properties of GABAergic currents and pharmacological properties, the localization and distribution of these receptors and their density, the connectivity of different interneurons, the uptake systems that control GABA levels, and the microglia and astrocytes that regulate extracellular ionic distributions. With regards to the connectivity of different types of interneurons, dendrites-targeted interneurons act by controlling the glutamatergic input of principal neurons and the generation of calcium currents, whereas somatic-targeting interneurons innervate hundreds to thousands of principal neurons leading to a synchronized activity in phase with the end of the synaptic current [19–22]. In addition, GABA and Glycine anionic receptor channel complexes have a unique property that is not shared by excitatory glutamatergic signals: the polarity of their actions depends largely on the levels of ongoing activity. Hyperactivity and a wide range of insults produce an accumulation of chloride ions leading to depolarizing and often excitatory effects of GABA [23–25]. Excitatory GABAergic signals can activate sodium channels, voltage-gated currents and remove the voltage-dependent blockade of NMDA receptor channels, leading to calcium influx with important long-lasting consequences on plasticity and network operation [23, 26–28].

The polarity of GABA actions also follows a developmental trajectory shifting from depolarizing/excitatory to hyperpolarizing/inhibitory in all animal species investigated [23, 29–32]. This is due to an evolutionary conserved progressive reduction of $[Cl^-]_i$ levels mediated by a decrease in the activity of a major chloride importer NKCC1 (Na-K-2Cl co-transporter isoform 1) and an increase of the chloride exporter KCC2 (K-Cl co-transporter isoform 2) activity [23, 25] (Fig. 1). During development GABAergic signals modulate cell proliferation, neuronal migration and growth as well as synapse and neuronal ensemble formation [23, 33–35]. The developmental stage at which the shift occurs is animal species, brain structure and sex specific [23]. In addition, there is an oxytocin-mediated neuroprotective transient shift to hyperpolarizing actions during parturition and birth [36, 37]. In experimental conditions, blocking the depolarizing actions of GABA by knocking out KCC2 or by in utero administration of NKCC1 antagonists produces deleterious effects including epilepsy and abnormal behavior in mice [17, 38–41].

Quite astonishingly, extensive investigations have unraveled a return to high $[Cl^-]_i$ levels and excitatory actions of GABA in many disorders and pathological conditions. This has been observed in rodent models of disorders that are generated in utero including Autism Spectrum Disorders (ASD), Fragile X, Rett and Down syndromes, maternal immune activation, various infantile epilepsies due to migration disorders, etc [12, 18, 41–53] Similar alterations are observed also in neurodegenerative disorders and adult insults or lesions including spinal cord injury, chronic pain, brain trauma, Parkinson's disease, Huntington's disease, deleterious actions of anesthetic agents, etc [12, 14, 54–62] High $[Cl^-]_i$ levels and a disruption of ionic equilibrium are also observed in brain tumors, notably neuro-glioblastoma [63–67]. The higher $[Cl^-]_i$ levels are due to a higher NKCC1/KCC2 activity ratio. Brain infarct also leads to similar changes with high $[Cl^-]_i$ levels and depolarizing/excitatory actions of GABA. Moderate to severe ischemic

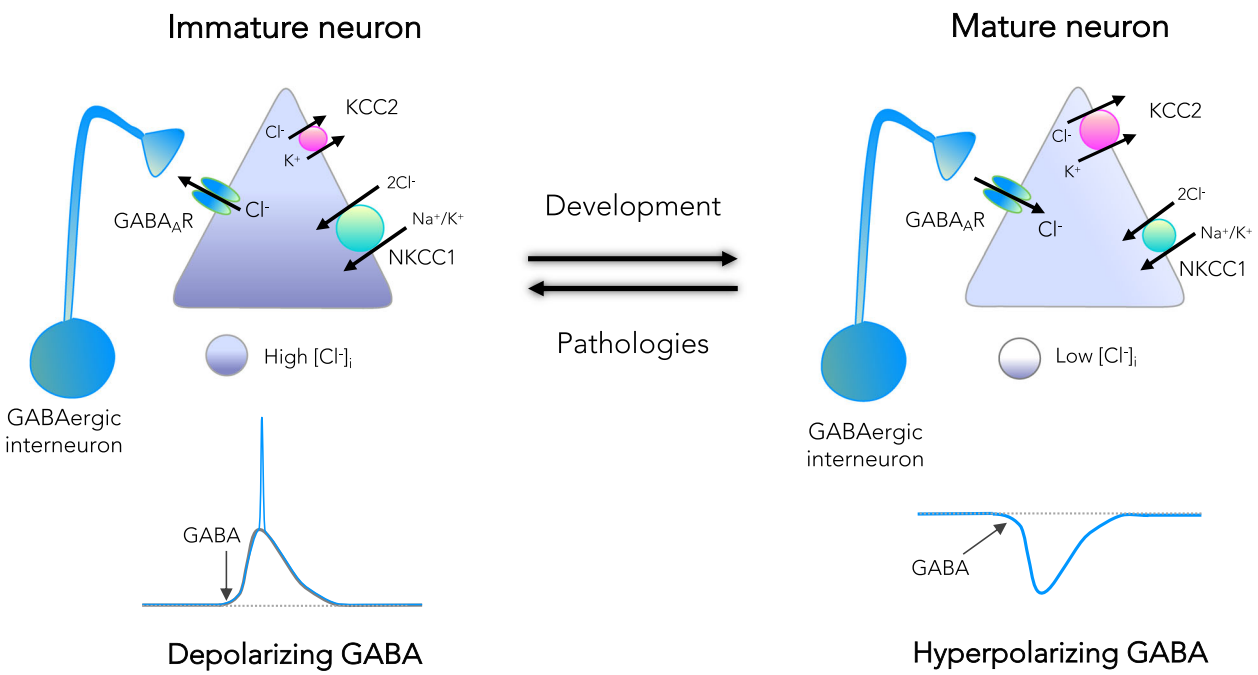

Fig. 1 Shifts in GABA polarity action in development and disease. Schematic representations of immature and mature neurons with GABAergic actions during development and its reversal in pathological conditions. Left: Immature neurons have a high intracellular chloride concentration ($[Cl^-]_i$) due to a high expression of NKCC1 and a low expression of KCC2; (bottom) activation of $GABA_A$ receptors by GABA application (arrow) results in a depolarization due to chloride efflux from the cell. Right: Mature neurons present low $[Cl^-]_i$ due to a low expression of NKCC1 and a high expression of KCC2; (bottom) activation of $GABA_A$ receptors by GABA application (arrow) results in a hyperpolarization. In pathological conditions there is a reversal to immature patterns with high $[Cl^-]_i$ and GABA-induced depolarization

conditions increase NKCC1 and/or reduce KCC2 activity [40, 68–75]. Therefore, in spite of the heterogeneity of these insults and their generating pathogenic event, they share a common reaction associated with high $[Cl^-]_i$ levels and depolarizing/excitatory actions of GABA (Fig. 1). Events underlying this shift include activation of kinases regulating NKCC1 and KCC2, and brain-derived neurotrophic factor released by microglia [40, 76–78]. Collectively, these observations illustrate the dynamic changes of GABA actions, the central role of the NKCC1/KCC2 ratio and their importance in the pathogenesis of infarcts and severe brain damage.

Restoring low $[Cl^-]_i$ levels and GABAergic inhibition has been shown to attenuate many brain disorders in experimental conditons and in clinical trials. The most frequently used agent to reduce $[Cl^-]_i$ levels is Bumetanide, a highly specific antagonist of the ubiquitously expressed NKCC1 chloride importer and the NKCC2 chloride co-transporter present in the thick ascending loop of Henle, hence its diuretic action. In animal models, Bumetanide attenuates the severity of ASD, Parkinson's disease, chronic pain, epilepsies, anesthesia induced seizures, etc [12, 71, 79–84] Furthermore, Bumetanide reduces ischemic infarction, cerebral swelling and neurological sequels in mice [71]. Successful clinical trials have also been made using Bumetanide with the aim of reducing $[Cl^-]_i$ levels to treat ASD [85–88] and related genetic syndromes with autistic features such as Tuberous Sclerosis [89]. Pilot trials also show a similar efficacy to treat Fragile X syndrome [90], schizophrenia [9] and Parkinson's disease [91]. Collectively, these studies suggest that the reduction of high $[Cl^-]_i$ levels and the shift of the polarity of GABA from excitation to inhibition might pave the way to innovative therapies of many disorders.

A working hypothesis: complementarity of Zolpidem and Bumetanide

Paradoxical actions of BZD are also observed after anesthesia where increasing doses of BZDs shift the effects from sedation to a paradoxical reaction with euphoria or dysphoria and purposeless movements [92]. How does Zolpidem and related agents produce these paradoxical reactions? Here, I propose that aberrant high $[Cl^-]_i$ levels and excitatory actions of GABA underlie the "awakening" produced by BZDs. In a comatose state, BZDs enhance the excitatory GABAergic activity leading to a *paradoxical transient awakening* instead of sleep and reduced activity. As such, this suggests that GABA exerts excitatory actions. Bumetanide might then restore low $[Cl^-]_i$ levels and efficient GABAergic inhibitory drive, decreasing the fundamental consequence of the initial trauma. Paradoxical effects of BZDs have been

demonstrated in experimental conditions when $[Cl^-]_i$ levels are high and the actions of GABA excitatory [41, 93, 94]. Phenobarbital also exerts paradoxical effects reducing early seizures but aggravating repeated ones when $[Cl^-]_i$ levels are increased [41]. In keeping with this, a pilot study reported that Bumetanide attenuated the severity of ASD and BZDs produced paradoxical reactions [95]. Collectively these observations raise the possibility that the paradoxical effects of BZDs are mediated by GABA excitatory actions that BZDs reinforce. Since $[Cl^-]_i$ levels cannot be determined in humans, these paradoxical actions of BZDs might justify the use of Bumetanide to reduce the potantially high $[Cl^-]_i$ levels, restore inhibition and attenuate the core apathic and akinetic syndrome.

Therefore, two agents acting differently on GABAergic networks, Zolpidem and Bumetanide, might emerge as potentially useful producing an awakening reaction. The former produces transient awakening effects that are not readily reproduced with repeated administration, the latter by reducing $[Cl^-]_i$ levels restores persistently GABA polarity and the efficacy of inhibitory networks. The use of Bumetanide is safe with minimal side effects even when administered for long periods, and it has been used for decades to treat hypertension and brain oedema with easily controlled side effects [96]. The administration of Bumetanide alone or possibly in combination with BZDs might therefore produce long term persistent awakening. This has been tested efficiently in a co-administration of BZD and Acetazolamide – a carbonic anhydrase inhibitor that reduces $[Cl^-]_i$ – showing an enhanced effect compared to the administration of BZD alone [60]. Also, Bumetanide enhances BZD efficacy in ischemic damage [13], and seizures are efficiently reduced by combined administration of Phenobarbital or BZDs and Bumetanide [94, 97]. Therefore, a dual drug administration has shown some efficacy in these pathologies.

Conclusion

In conclusion, paradoxical actions of BZDs can be viewed as a clinical signal reflecting a disturbance of the regulation of $[Cl^-]_i$ levels and the polarity of GABA. It is therefore suggested that a similar mechanism might operate in these patients. This also suggests that Bumetanide, known in experimental and clinical situations (pilot cases) to reduce/attenuate the severity of an insult, might be useful to correct the fundamental cause of the disorder. Clearly, low $[Cl^-]_i$ levels constitute a general signature of insults that must be treated by restoring the correct polarity of GABA actions.

Abbreviations

ASD: Autism Spectrum Disorders; BZDs: Benzodiazepines; EEG: Electroencephalogram; GABA: Gamma-aminobutyric acid; KCC2: K-Cl co-transporter isoform 2; NKCC1: Na-K-Cl co-transporter isoform 1

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