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How chronic administration of benzodiazepines leads to unexplained chronic illnesses: A hypothesis

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A B S T R A C T

It is thought that an ill defined biochemical cascade may lead to protracted withdrawal symptoms subsequent to discontinuance of routine use of benzodiazepine class drugs and establish chronic illness in some patients. In this review, published findings are presented that support the novel concept that withdrawal from benzodiazepine class drugs can trigger elevated and sustained levels of a potent oxidant called peroxynitrite via potentiation of the L-type voltage-gated calcium channels, and in the later stages of withdrawal, via excessive N-methyl-D-aspartate receptor activity, as well. Potentiation of L-type voltage-gated calcium channels and excessive N-methyl-D-aspartate receptor activity both result in calcium influx into the cell that triggers nitric oxide synthesis. In pathophysiological conditions, such increased nitric oxide synthesis leads to peroxynitrite formation.

The downstream effects of peroxynitrite formation that may occur during withdrawal ultimately lead to further peroxynitrite production in a system of overlapping vicious cycles collectively referred to as the NO/ONOO(−) cycle. Once triggered, the elements of the NO/ONOO(−) cycle perpetuate pathophysiology, perhaps including reduced $GABA_A$ receptor functioning, that may explain protracted withdrawal associated symptoms while the vicious cycle nature of the NO/ONOO(−) cycle may explain how withdrawal becomes a chronic state.

Suboptimal levels of tetrahydrobiopterin may be one risk factor for the development of the protracted withdrawal syndrome as this will lead to partial nitric oxide uncoupling and resultant peroxynitrite formation. Nitric oxide uncoupling results in superoxide production as calcium-dependent nitric oxide synthases attempt to produce nitric oxide in response to L-type voltage-gated calcium channel-mediated calcium influx that is known to occur during withdrawal. The combination of nitric oxide and superoxide produced, as when partial uncoupling occurs, react together in a very rapid, diffusion limited reaction to form peroxynitrite and thereby trigger the NO/ONOO(−) cycle.

The NO/ONOO(−) cycle may explain the nature of the protracted withdrawal syndrome and the related constellation of symptoms that are also common in other illnesses characterized as NO/ONOO(−) disorders such as myalgic encephalomyelitis/chronic fatigue syndrome and fibromyalgia.

Introduction

Benzodiazepines are a class of drugs that are prescribed for treating a wide variety of disorders, including various forms of anxiety disorders, epilepsy, muscle spasms, insomnia, and tinnitus. Benzodiazepines are sometimes characterized as “minor tranquilizers,” due to their potentially sedating effect. Benzodiazepines are thought to exert their anxiolytic effect by enhancing the activity of the inhibitory neurotransmitter, GABA (gamma-aminobutyric acid). Among the most prescribed benzodiazepine drugs are clonazepam, brand name Klonopin, alprazolam, brand name, Xanax, lorazepam, brand name Ativan, and diazepam, brand name, Valium. A major drawback to benzodiazepines, however, is that long term use, including normal therapeutic dosing, can lead to the development of physical dependency and withdrawal symptoms upon discontinuance [1]. For those who use benzodiazepines at therapeutic doses, benzodiazepine dependence is primarily concerned with the negative subjective effects of withdrawal upon discontinuance and not reward-seeking effects of the benzodiazepine use [2].

Observations that benzodiazepine withdrawal associated symptoms can be severe and protracted

According to physician and Emeritus Professor of Clinical Psychopharmacology at Newcastle University, Heather Ashton, it may be appropriate to characterize the constellation of symptoms associated

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with withdrawing from benzodiazepine use as an illness rather than simply "withdrawal" in cases in which symptoms persist for a period of several months to several years after discontinuance.

> "The features of benzodiazepine withdrawal appear to constitute a new syndrome characterised by a particular cluster of symptoms and a protracted clinical course…In addition the course of the benzodiazepine withdrawal syndrome appears to be much longer than that of other drugs of dependence, and in particular longer than that reported for benzodiazepines, which has been stated to last 5–15 days, 2–4 weeks, and 10–54 days" [3].

Malcolm Lader, Emeritus Professor at the Institute of Psychiatry, Psychology, and Neuroscience at King's College in London, echoed Ashton's observations in both his research and public comments. In a radio interview, Lader stated that a subset of patients experience long-term withdrawal lasting two or more years with some reports of patients still experiencing symptoms ten years after discontinuance [4].

Of those that suffer from a protracted withdrawal, most recover from associated anxiety and depression long before resolution of physical symptoms such as muscle spasm [5]. Ashton listed several common "somatic" withdrawal symptoms which she distinguished from the common psychiatric symptoms associated with withdrawal, such as anxiety [3]. Among the somatic symptoms, Ashton noted paresthesia, pain and stiffness in various parts of the body, tremors in hand and jaw, muscle fasciculation, myoclonic jerk, ataxia, visual disturbances, photophobia along with increased sensitivities to noise, taste and smell, gastrointestinal disturbances, "influenza-like" symptoms including weakness, postural dizziness, aches and pains, but not accompanied by fever [3]. Among common withdrawal symptoms, Brett and Murnion noted tinnitus along with many other symptoms, including several that are identical to the ones reported by Ashton [6]. While many of the withdrawal symptoms experienced by patients may be similar in nature to their symptoms for which the drug was originally prescribed [6], old symptoms often return with pronounced severity, and indeed, new symptoms commonly emerge in withdrawal never before experienced by the patient [5]. Additionally, Pittman et al. conducted a survey of 493 self-selected participants from the benzodiazepine withdrawal support website, Benzobuddies.org, indicating the cluster of symptoms experienced by withdrawal sufferers was consistent notwithstanding the symptoms the participant experienced prior to benzodiazepine use [7]. Most interestingly, when comparing participants with no prior psychiatric history to those with a history of psychiatric disorder, Pittman et al. found no significant differences in reporting of psychiatric symptoms (with the exception of suicidal and panic symptoms) experienced during withdrawal [7]. Of the 493 surveyed, 156 participants reported that they had discontinued benzodiazepine use and that their withdrawal symptoms had subsided [7]. The average length of time that withdrawal symptoms persisted *after withdrawal from the drug was complete* for these 156 participants was 14 months [7].

Benzodiazepine Information Coalition (BIC), a patient-driven non-profit organization that raises awareness of the risks associated with benzodiazepine administration, reports a wide range of common symptoms associated with withdrawal, including those observed by Ashton, Lader and Pittman, as well as the potential for withdrawal symptoms to persist for a period of several months or even several years [8,9]. While the epidemiology of protracted withdrawal syndrome (PWS) has not been rigorously studied, based on informal patient surveys and reporting, Ashton and BIC agree the number who are severely affected in the long term to comprise a significant minority, perhaps representing somewhere between 10% and 15% of all chronic benzodiazepine users [5,8]. Given the astounding number of prescriptions for benzodiazepines, this significant minority might represent upward of over one million individuals in the United States alone. Emergence of PWS from chronic use of benzodiazepines appears to be independent of dosing [2,5,8]. To date, there has been no detailed proposal for how PWS is initiated and perpetuated and what factors predispose one to PWS upon discontinuance from routine use of benzodiazepines.

Background: prior research concerning pathophysiological effects of benzodiazepine sensitization & withdrawal

Research has demonstrated why benzodiazepines tend to lose their effectiveness with chronic use over time and lead to physical dependency. Essentially, GABAergic function is diminished after chronic benzodiazepine treatment [10], and up-regulation of glutamatergic neurotransmission occurs, although resultant glutamatergic over-activity may not be observed until subsequent to withdrawal [11]. After withdrawal occurs, glutamatergic over-activity is no longer masked by the benzodiazepine's heightened inhibitory effect on the GABAergic system [11].

Previously studied effects of benzodiazepine withdrawal on L-type voltage gated calcium channels

Benzodiazepines' action on voltage activated calcium channels appear to play an important role in benzodiazepine dependency and withdrawal [12]. Benzodiazepine administration can regulate L-type voltage-gated calcium channel (L-type VGCC) expression [12]. Benzodiazepines can directly inhibit L-type VGCC-mediated calcium influx and high voltage-activated (HVA) currents [13–15]. Benzodiazepines' inhibition of L-type VGCC's may partially explain the therapeutic effect of clonazepam, a benzodiazepine class drug, in the treatment of ME/CFS, in lowering over-activity of the brain [16].

Benzodiazepines, however, can have a paradoxical effect on L-type VGCC expression, especially during withdrawal [12]. Xiang et al. cited the finding that [^{3}H]diltiazem (a calcium channel blocking drug) binding sites were up-regulated in mouse cortical cultures with concomitant increase in L-type VGCC subunit expression after 3-day exposure of mouse cortical cultures to 1,4- or 1,5-benzodiazepines and in diazepam-treated mice exhibiting withdrawal signs [17] as part of the foundation for their experiment that revealed "a doubling of HVA calcium current density was detectable [in rat hippocampal CA1 pyramidal neuron cell cultures] immediately after ending 1-week flurazepam (a benzodiazepine) administration and was sustained for at least 2 days after ending treatment before tapering off 3 days after treatment" [12].

Summarizing the role of L-type VGCCs in withdrawal, Xiang et al. states:

> "The accumulated evidence supports the possibility that L-type VGCCs may be a source for diverse intracellular messengers or transcription factors to increase glutamatergic strength during benzodiazepine withdrawal and contribute to benzodiazepine physical dependence" [12].

Role of AMPA receptors in benzodiazepine withdrawal

α-amino-3-hydroxy-5-methylisoxasole-4-propionic acid (AMPA) receptors appear to play a significant role in the initial stages of benzodiazepine withdrawal pathophysiology [11]. AMPA receptor mediated mEPSC amplitude increased an additional 30 to 50% in 2-day flurazepam-withdrawn rats; however, pretreatment with the L-VGCC antagonist, nimodipine, abolished the transient increase in AMPAR-current potentiation, demonstrating L-VGCC's role in benzodiazepine dependence and withdrawal [12]. Such AMPAR current enhancement is linked to severity of withdrawal anxiety [18], at least during the early stages of withdrawal.

Role of NMDA receptors in benzodiazepine sensitization and withdrawal

While N-methyl-D-aspartate (NMDA) receptor EPSC amplitude has been found to be significantly decreased in hippocampal slices

following chronic benzodiazepine treatment [19], that observation may be limited in scope to the early withdrawal stages as "the AMPA receptors seem to be crucial in initiation of the withdrawal symptoms, whereas the NMDA receptors play an important role during the later stage" [11,20]. NMDA receptors are not only involved in the later stages of withdrawal, they also appear to play a significant role in sensitization to benzodiazepines during use of the drug, whereas AMPA receptors appear to be activated in the withdrawal process only [11,20]. Administration of NMDA receptor antagonists prevented the development of tolerance and/or expression of tolerance to the motor impairing effect of diazepam indicating the role of excessive NMDA receptor activity in benzodiazepine sensitization or drug tolerance [21]. Talarek et al. further elucidated the role of excessive NMDA activity in benzodiazepine sensitization by observing the statistically significant therapeutic effect of the NMDA-antagonists, memantine and ketamine, in attenuating pentylenetetrazole-induced death incidents, tonic seizures, and clonic seizures in two groups of diazepam-sensitized mice: one group administered diazepam for 21 consecutive days and the other administered diazepam for three 7-day periods that were interspersed with 3-day periods during which they received saline injections [11]. Interestingly, "both the number of convulsions and death episodes were significantly higher in mice treated in the scheme with diazepam-free periods, as compared to the control group given the benzodiazepine for 21 straight consecutive days" evidencing how repeated withdrawal episodes leads to subsequently worsening withdrawal episodes and perhaps presenting a model of withdrawal-induced "kindling."

Kindling occurs when an organism is exposed repeatedly to an initially sub-threshold stimulus resulting in hypersensitivity and spontaneous seizure-like activity [16]. The implication of kindling within the context of benzodiazepine administration is that individuals who have had previously endured one or more withdrawal episodes are at increased risk of the subsequent withdrawal being more severe [8].

Previously studied effects of benzodiazepine withdrawal on $GABA_A$ receptors and $GABA_A$ receptor mediated current

Chronic administration of benzodiazepines appears to down-regulate $GABA_A$ receptor function in multiple ways. Receptor down-regulation involves a decrease in the number of receptor sites [22], reductions in benzodiazepine receptor and GABA receptor allosteric coupling that vary from one brain structure to another [23], and modulation of $GABA_A$ receptor subunit messenger RNA (mRNA) and protein subunit expression [24]. Benzodiazepine-induced modulation of $GABA_A$ receptor subunit mRNA and resultant changes in protein subunit expression may be linked to increases in intracellular calcium and calcium/calmodulin-dependent protein kinase or phosphatases signaling mechanisms [25]. Changes in GABA receptor activity in one brain region could lead to downstream effects in interconnected brain structures not immediately impacted by benzodiazepine-induced receptor changes [23].

In addition to increasing glutamatergic strength, L-type VGCC activation during withdrawal also leads to significantly reduced $GABA_A$ receptor-mediated synaptic transmission in CA1 pyramidal neurons *in vivo* and *in vitro* [25]. Such findings are supported by the demonstration that administration of the L-type VGCC antagonist, nimodipine, abolished the reduction in $GABA_A$ receptor current in hippocampal cell cultures in rats 2 days withdrawn from flurazepam [25]. Essentially, benzodiazepine-mediated $GABA_A$ receptor down-regulation can occur independent of the effects of reductions in allosteric coupling between benzodiazepine and GABA binding sites [25]. Again, such down-regulation in $GABA_A$ receptor current appears to arise via calcium-dependent phosphorylation/dephosphorylation downstream of L-type VGCC activation "suggesting that intracellular calcium homeostasis is important to maintain $GABA_A$ receptor function" [25].

There also may be interplay between $GABA_A$ receptor down-regulation and enhanced NMDA receptor activity. A potential downstream effect of lowering $GABA_A$ receptor function is elevated NMDA receptor activity [26]. Excessive NMDA receptor activity is associated with benzodiazepine sensitization during administration of the drug *and* in the later stages of withdrawal, [11,20], therefore, excessive NMDA activity would then become a significant source for increases in intracellular calcium along with L-type VGCC activation. Calcium influx through NMDA receptor channels can result in long-term suppression (or potentiation, depending on the amount of intracellular calcium [27]) of $GABA_A$ receptor function in CA1 pyramidal cells similar to L-type VGCC calcium influx [28]. Therefore, lowering $GABA_A$ receptor function enhances NMDA receptor activity, and enhanced NMDA receptor activity can, in turn, lower $GABA_A$ function via elevations in intracellular calcium and resultant changes in mRNA and protein subunit expression.

Previous studies of benzodiazepine sensitization and withdrawal do not articulate how withdrawal can become a chronic state

Variation in reduction of GABAergic functioning and perhaps concomitant enhancement of NMDA receptor and L-type VGCC expression from patient-to-patient in response to routine benzodiazepine administration likely explain differences in development of tolerance during benzodiazepine use and withdrawal severity upon discontinuance. Even in cases of particularly intense withdrawal marked by substantially elevated HVA calcium currents and AMPA receptor mediated currents, the affected tissues, however, may return to baseline in as little as a matter of days as reported in the Xiang et al. laboratory studies via some normalizing response, perhaps the cyclic guanosine monophosphate (cGMP) – protein kinase G pathway. So what might make acute withdrawal from benzodiazepine discontinuance, a potentially dangerous but typically transitory event lasting up to a few weeks, become a protracted illness lasting several months or years?

Hypothesis:. how withdrawal from benzodiazepines might trigger chronic illness: proposed mechanism by which withdrawal becomes protracted

While research of benzodiazepines has demonstrated their limitations due to their tendency to result in development of tolerance during use and emergence of withdrawal symptoms upon discontinuation, these studies do not capture the genesis and propagation of a chronic illness process observed in patients with PWS. While many in the medical community are aware of the dangers that withdrawal presents in the acute stages, very few are aware that pathophysiological events during withdrawal are a possible trigger for chronic illness. For example, the Xiang et al. studies report that calcium current density and AMPA receptor activity in rat derived hippocampal cultures returned to baseline in 3–4 days after withdrawal [12]. Such reporting may belie the potential of withdrawal to result in long-term illness or disability as seen in persons with PWS. This work, of course, did not assess PWS given its timeframe of paradigm for both administration and follow-up by recording. In the Xiang et al. and similar studies, return to baseline of neural cultures in a matter of a few days may be in part due to the relatively short duration of benzodiazepine administration in laboratory animals, but it may also be due to neglecting to test certain vulnerability in subjects as will be further discussed.

Increases in intracellular calcium can lead to peroxynitrite formation especially when BH4 is low

The increased intracellular calcium produced by VGCC activation, and in particular L-type VGCC activation, as has been reported to occur in benzodiazepine withdrawal [12], may lead to multiple regulatory responses, including increased nitric oxide levels produced through the action of the two calcium/calmodulin-dependent nitric oxide synthases, neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide

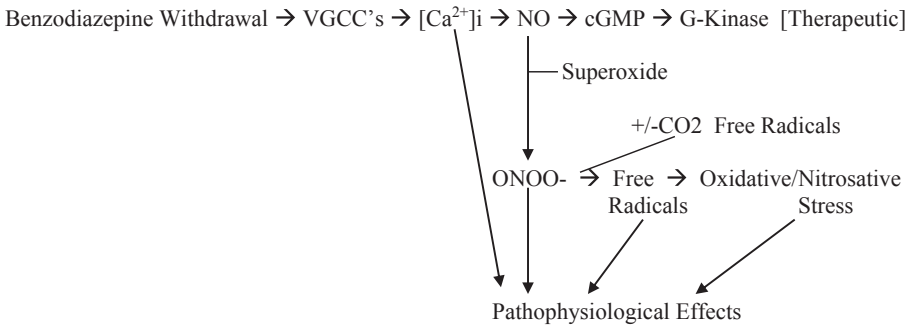

Fig. 1. The benzodiazepine withdrawal – peroxynitrite pathway [31].

synthase (eNOS) [29–31]. While activation of other types of VGCC's may be implicated in benzodiazepine withdrawal, L-type or Long Lasting VGCC's are significant because once activated, they remain open a fairly long time allowing for greater calcium influx [30]. eNOS and nNOS are almost completely inactive when calcium levels are low in the cell, but with increase in calcium, eNOS and nNOS generate huge amounts of nitric oxide [31]. While increased nitric oxide levels typically act in the therapeutic context of increased synthesis of cyclic guanosine monophosphate (cGMP) and subsequent activation of protein kinase G, in most pathophysiological contexts, nitric oxide reacts with superoxide to form peroxynitrite (ONOO−), which can produce radical products, including hydroxyl radical (˙OH) and carbonate radical (CO_3^{-}) [29,32].

Consistent with nitric oxide playing a pathophysiological role in withdrawal, the nitric oxide synthase inhibitors, 7-nitroindazole (7-NI) and methylene blue at doses of 5.0 and 10.0 mg/kg, significantly lowered incidents of pentylenetetrazole-induced tonic seizure, clonic seizure and death, while NG-nitro-L-arginine methyl ester (L-NAME) at the same doses lowered incident of tonic seizure, but not clonic seizure or death, in diazepam-sensitized mice during diazepam-free intervals [11]. Interpreting these findings, Talarek et al. implicated the NMDA receptor – nitric oxide – cGMP pathway in benzodiazepine sensitization pathophysiology [11]; however, they did not rule out the possibility that the therapeutic effect of the nitric oxide lowering agents used in their experiment could be attributable to their role in lowering peroxynitrite formation. All of the agents used in their experiment to lower the nitric oxide – cGMP pathway, including methylene blue [33–34], inhibit nitric oxide synthesis and presumably inhibit incidental peroxynitrite formation, as well. Furthermore, the nitric oxide – cGMP pathway cannot be presumed to necessarily have pathophysiological consequence in the context of benzodiazepine sensitization and withdrawal because this pathway is known to be tissue protective in several contexts. While up-regulation of the soluble guanylyl cyclase – cGMP in dopamine-depleted striatum may contribute to enduring changes in neuronal excitability in Parkinson's Disease [35], an observed pathophysiological role, in other contexts, cGMP was found to inhibit neurotoxicity induced by H_2O_2 [36], prevent kainite-induced oligodendrocyte-like cell excitotoxicity by decreasing kainite-induced calcium influx [37], and increase expression of the cytoprotective gene, heme oxygenase-1 [38].

So what might tip the scales in favor of nitric oxide synthesis resulting in peroxynitrite formation? Tetrahydrobiopterin (BH4) deficiency, low BH4 to dihydrobiopterin (BH2) ratios and resultant partial nitric oxide uncoupling may be such vulnerability [39–41]. BH4 is an essential co-factor in NOS-catalyzed nitric oxide biosynthesis [39,42]. A deficiency in BH4 triggers peroxynitrite generation in the NOS catalyzed reaction [39,41,43]. Peroxynitrite gets formed during nitric oxide synthesis because electron transfer from all three nitric oxide synthases, nNOS, eNOS, and iNOS, become "uncoupled" from L-arginine oxidation when BH4 levels are insufficient, thereby producing superoxide in place of nitric oxide [41,43]. Partial uncoupling, therefore, will lead the nitric oxide synthases to act like peroxynitrite synthases [41,43].

Intracellular calcium-mediated peroxynitrite formation independent of nitric oxide uncoupling

Significant increases in intracellular calcium, as occurs in withdrawal, can also lead to increased superoxide formation independent of nitric oxide uncoupling via resultant increases in intramitochondrial calcium and its effect on the electron transport chain [44], NADPH oxidase which is mediated through a calcium-dependent mechanism [44–46], and xanthine oxidase, also increased by a calcium-dependent mechanism [47,48]. Both NADPH oxidase and xanthine oxidase produce superoxide as a product [44]. Resultant superoxide will react with nearby nitric oxide to form peroxynitrite.

The withdrawal-induced pathophysiological cascade

Fig. 1 is a diagram adapted from the one Pall used in his presentation on electromagnetic field exposure for purposes here of illustrating proposed events downstream of benzodiazepine withdrawal [note: the diagram below is identical to Pall's with the exception that "Benzodiazepine Withdrawal" is placed in lieu of microwave/low frequency electromagnetic fields (EMF's)]:

The NO/ONOO(−) cycle

According to Martin Pall, Emeritus Professor of biochemistry at Washington State University, the above pathophysiological effects can become chronic because once peroxynitrite is elevated, there are feedback mechanisms that can maintain elevated levels of peroxynitrite in a model Pall dubbed the NO/ONOO(−) cycle [47,49]. The "NO" in NO/ONOO(−) cycle is the abbreviation for nitric oxide, and "ONOO(-)" is the abbreviation for peroxynitrite. Probably the best way to begin a discussion of Pall's NO/ONOO(−) cycle is to present the diagram that Pall uses in his publications and presentations to illustrate the elements and multilayered nature of these vicious cycles. Most of the mechanisms involved are well accepted biochemistry and physiology and so the novelty of the theory lies in the way in which Pall elucidated that the various elements involved form interacting vicious cycles [49].

[30,49]. Increasing any of the cycle elements illustrated above, i.e. a short-term stressor, can initiate the NO/ONOO(−) self-perpetuating cycle, and the multiple interacting vicious cycles therein explain the chronic nature of NO/ONOO(−) cycle diseases [49].

As depicted in the illustration, nitric oxide and superoxide react in a very rapid, diffusion limited reaction to form peroxynitrite (OONO−) [47]. Again, nitric oxide is synthesized by three different isozymes of nitric oxide synthase, two of which are constitutive, eNOS and nNOS and one of which is highly inducible, iNOS [47]. Of the nitric oxide synthases, iNOS has the central role in initiating the NO/ONOO(−) cycle when the cycle is triggered by viral or bacterial infection [47] while the two constitutive synthases, eNOS and nNOS, have the central

role in initiating the cycle in PWS, analogous to their role in electromagnetic hypersensitivity downstream of VGCC activation and elevated intracellular calcium [29]. Once the NO/ONOO(−) cycle is initiated, however, all three nitric oxide synthases' relative roles in propagating the cycle may become quite similar in perpetuating the cycle. The significance here is that if so-called mystery maladies are, at least in part, NO/ONOO(−) disorders, they can be initiated by means other than viral or bacterial trigger.

Nitric oxide formed by the calcium-dependent constitutive nitric oxide synthases, eNOS and nNOS, can combine with superoxide to generate peroxynitrite. Again, as previously discussed, partial nitric oxide uncoupling will lead to higher production of peroxynitrite during NO synthesis. This problem becomes compounded as peroxynitrite further oxides and depletes BH4 in a reciprocal relationship that Pall calls the "central couplet" of the NO/ONOO(−) cycle [49]. Peroxynitrite and superoxide can cause mitochondrial dysfunction and lead to depletion of energy, ATP [49]. ATP depletion can lead to partial depolarization of the plasma membrane, which can increase susceptibility of the NMDA receptors to stimulation or alternatively activate VGCC channels [30,50]. The NMDA receptors and VGCC's work in parallel in the NO/ONOO(−) cycle as activity of both result in increased intracellular calcium, "so the downstream effects of the NMDA receptors and the VGCC's are very similar" [30].

Elevated levels of peroxynitrite produce oxidative stress, and both can induce the transcription factor NF-kappaB, increasing production of inflammatory cytokines, and in turn, induce iNOS to form more nitric oxide [49]. Also, several of the transient receptor potential cation channels, or TRP receptors, are susceptible to oxidative stress and can get activated by oxidants, and, in turn, produce elevated levels of intracellular calcium [30]. Pall has enumerated other means by which cytoplasmic calcium becomes and remains elevated by virtue of the NO/ONOO(−) cycle elements, such as ATP depletion resulting in lowered ability to pump out calcium via the calcium pump ATPase (indicated by the arrow in Fig. 2a from "ATP" to "[Ca^{2+}]i") and peroxynitrite-induced peroxidation of the plasma membrane allowing increased calcium influx from the surrounding medium [47]. Additionally, it is known that oxidants increase intracellular calcium release from the endoplasmic reticulum [51] introducing more calcium to the two calcium-dependent constitutive nitric oxide synthases, eNOS and nNOS, in the cytosol, prompting further nitric oxide synthesis.

The NO/ONOO(−) cycle and $GABA_A$ receptor function

In addition to all the mechanics of the NO/ONOO(−) cycle that Pall has set forth, it is proposed here that an additional effect may be connected to the cycle: down-regulation or reduced $GABA_A$ receptor function. As previously discussed, research has demonstrated that $GABA_A$ receptor functioning may be dependent on proper calcium homeostasis and showed that elevated calcium influx through L-type VGCC's [25] and elevated calcium influx through the NMDA pore [28] both led to lower $GABA_A$ receptor functioning. And although only larger rises of intracellular calcium lower $GABA_A$ receptor functioning, whereas smaller rises have the opposite effect by potentiating $GABA_A$ receptors [27], the NO/ONOO(−) cycle may result in levels of intracellular calcium sufficient to inhibit $GABA_A$ receptor functioning. As discussed above, there are a number of pathways by which peroxynitrite can directly or indirectly elevate and sustain elevated levels of intracellular calcium. Because the NO/ONOO(−) cycle maintains elevated levels of intracellular calcium, $GABA_A$ receptor functioning may be impaired for as long as the NO/ONOO(−) cycle propagates itself. The NO/ONOO(−) cycle is illustrated again in Fig. 2b. below but with an additional arrow pointing out reduced $GABA_A$ receptor functioning in relation to the existing cycle elements.

Lowered $GABA_A$ receptor function may not only be a consequence of the NO/ONOO(−) cycle, it may also initiate, and perhaps play a role in perpetuating the cycle by resulting in elevated NMDA receptor activity [26]. In his paper on Multiple Chemical Sensitivity, Pall noted that a potential downstream effect of lowering $GABA_A$ receptor function is elevated NMDA receptor activity [26]. Conversely, enhancing $GABA_A$ receptor function inhibits the NMDA/NO/cGMP pathway [52]. If down-regulation of the $GABA_A$ receptor is linked to the NO/ONOO(−) cycle, then such effect has consequence for all NO/ONOO(−) cycle illnesses.

Hypoxia, BH4 and peroxynitrite formation

Hypoxia may be another potential pathway into NO/ONOO(−) cycle pathophysiology. BH_4 deficiency in hypoxia triggers peroxynitrite production [39] consistent with Pall's NO/ONOO(−) theory. It should be noted that Delgado-Esteban et al. focused on mitochondrial dysfunction as the source of reactive oxygen species (ROS) and peroxynitrite formation in response to ATP depletion in hypoxia [39]; however, calcium influx from L-type VGCC activation may be another trigger for

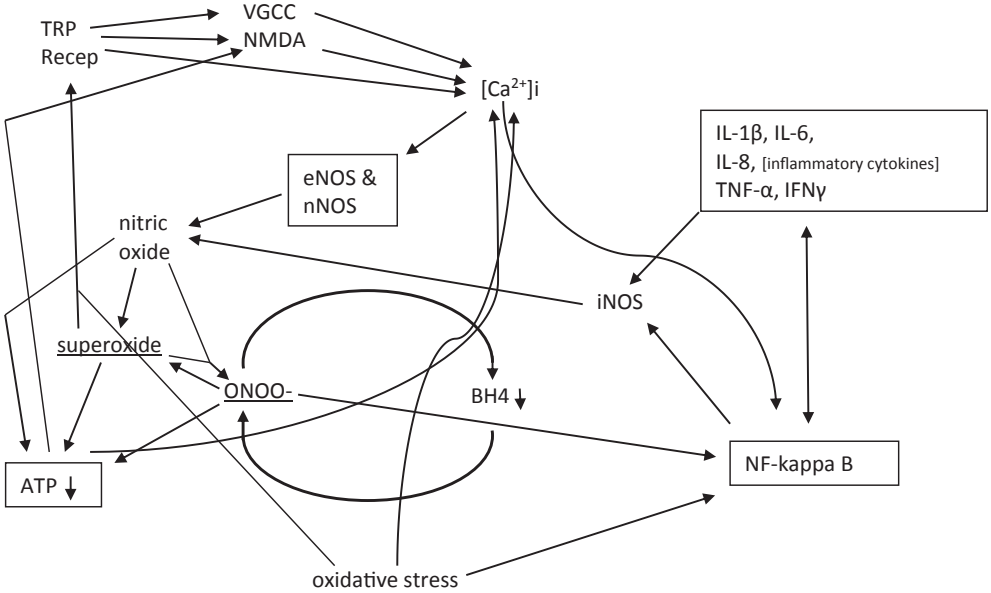

Fig. 2a. The NO/ONOO(−) cycle.

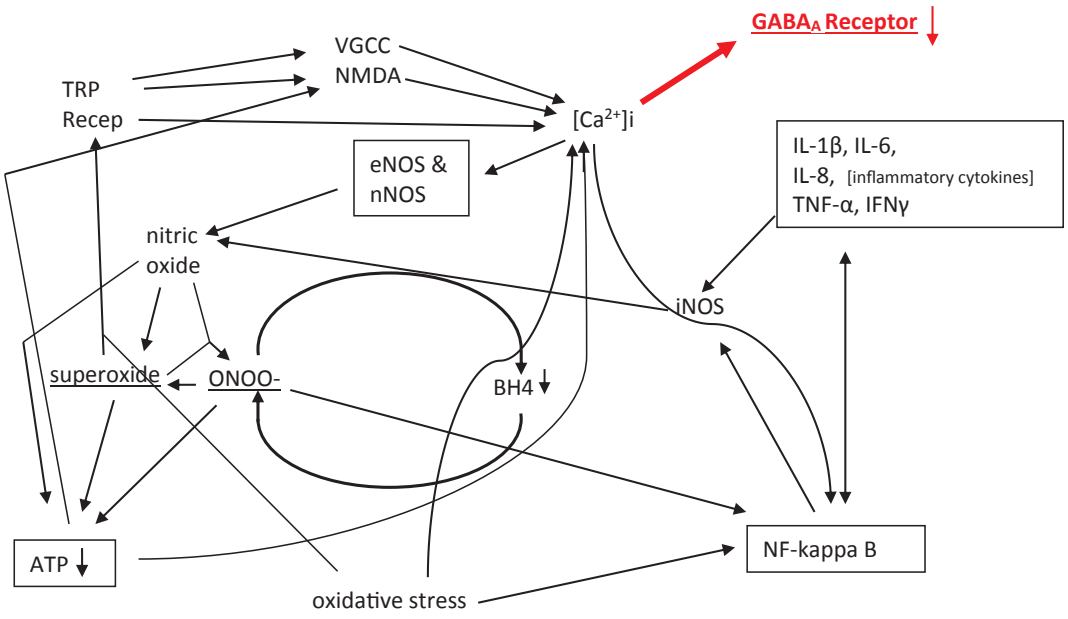

Fig. 2b. The NO/ONOO(−) cycle effect on GABA$_A$ receptor functioning.

peroxynitrite formation in hypoxia as hypoxia transiently potentiated L-type VGCC currents with HVA calcium current density increased 1.5-fold [53]. While Xiang et al. focused on the role of calcineurin in potentiation of VGCC's in hypoxia [53], it is known that a drop in ATP concentration triggered by anoxic, and presumably hypoxic, conditions inhibits the plasma membrane leading to depolarization in an event called anoxic depolarization [50]. Again, consistent with Pall's NO/ONOO(−) cycle theory, such depolarization can lead to activation of VGCC's or alternatively excessive NMDA activity [30].

Protracted withdrawal syndrome in the context of other neuro-dysfunctional disorders

Many of the protracted withdrawal symptoms are commonly associated with so-called "mystery" illnesses such as myalgic encephalomyelitis/chronic fatigue syndrome, (ME/CFS), fibromyalgia (FM), and postural orthostatic tachycardia syndrome (POTS) suggesting overlapping etiology. However, while some persons with PWS have complained of symptoms that emerged during withdrawal that are identified as being hallmark symptoms of ME/CFS (post-exertion fatigue), FM (chronic pain with associated trigger points) or POTS (intolerance to standing), more common is that persons with PWS complain of symptoms commonly associated with these illnesses, although not hallmark symptoms, such as gastrointestinal disturbances, clouding of consciousness commonly referred to as brain fog, and photophobia [3,9]. In addition to similarity in symptomology, patients suffering from PWS often report having markedly low functioning such as is common with ME/CFS, FM, and POTS [8]. The course of PWS versus ME/CFS, FM, and POTS, however, may be somewhat different. Even in cases of protracted withdrawal, symptoms typically resolve, although remission may take several years [54], whereas rates of recovery in ME/CFS, FM and POTS are likely lower.

Mystery ailments including PWS may at least in part be NO/ONOO(−) cycle illnesses

Pall has characterized ME/CFS, FM, post-traumatic stress disorder (PTSD), tinnitus and other poorly understood illnesses as NO/ONOO(−) disorders [47,49,55]. Peroxynitrite can be measured by its decomposition product, 3-nitrotyrosine [32]. ME/CFS patients have very high levels of peroxynitrite as measured by 3-nitrotyrosine, averaging 5.43 times the levels of healthy controls, with no overlap with controls [56]. Peroxynitrite's central pathogenic role has also been proposed in stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders [32,44]. Interestingly, heart failure appears to have the most evidence to support its characterization as a NO/ONOO(−) cycle disease [44]. Note that in heart failure, proposed NO/ONOO(−) cycle pathophysiology is local to cardiac tissues [44].

Besides the vicious cycle nature of the NO/ONOO(−) cycle, another central tenant of the NO/ONOO(−) cycle is that the basic mechanism is local and will be localized to different tissues in different individuals due to the limited half-lives of the three compounds involved, NO, superoxide, and peroxynitrite [49]. Pall attributes the variations of symptoms between patients with disorders such as ME/CFS and FM to the local nature of the NO/ONOO(−) cycle [49].

The heterogeneity of symptoms associated with the mystery illnesses has confounded medicine, and PWS appears to be no different in its scope of wide varying symptoms from one patient to another. The local nature of the NO/ONOO(−) cycle might shed much light on this phenomenon. Given the wide variation of function of one part of the central nervous system (CNS) contrasted to another, it is readily conceivable how impairment by NO/ONOO(−) cycle pathology of one part of the CNS, for example the hippocampus, could result in different symptoms than impairment of the thalamus. Under the NO/ONOO(−) cycle theory, the variation of symptomology in these illnesses is less surprising when also factoring potential dysfunction of any particular part or parts of the peripheral nervous system (PNS), for example the NO/ONOO(−) cycle occurring in the vagus nerve. Evidence indicates benzodiazepines have similar inhibitory effects on the PNS as the CNS [57,58] and VGCC's are also found in the PNS [59]. Conceivably benzodiazepine's effect on enhancing gabaergic signaling in the PNS could result in compensatory GABA$_A$ receptor down-regulation and increased L-type VGCC expression in the PNS during withdrawal.

ME/CFS, FM, and POTS may be characterized as neuro-dysfunctional disorders. Indeed, various forms of dysautonomia commonly coexist with ME/CFS and FM, and dysautonomia is the hallmark of POTS [60,61]. A number of the symptoms associated with PWS following chronic administration of benzodiazepines indicate the presence of dysautonomia, and it is the objective of this paper to set forth the hypothesis that PWS is a neuro-dysfunctional disorder marked by elevated levels of peroxynitrite and perpetuated by the NO/ONOO(−)

Table 1
Predictions in PWS.

Expected Findings	Rationale
The amount of peroxynitrite formed in a patient subsequent to withdrawal is a predictor for the development and level of severity of PWS.	The more peroxynitrite formed during withdrawal, the stronger the cascade of downstream effects will be in perpetuating the NO/ONOO(−) cycle and concomitant withdrawal symptoms.
Peroxynitrite levels subsequent to withdrawal, as measured by nitrotyrosine, are highest in patients with the lowest levels of BH4.	During withdrawal, significant increases in $[Ca^{2+}]i$ from L-type VGCC activation result in increased nitric oxide production. As calcium-dependent eNOS and nNOS produce nitric oxide in response to L-type VGCC-mediated Ca^{2+} influx, deficiency of BH4 will lead to increased superoxide formation due to partial nitric oxide uncoupling. The nitric oxide and superoxide produced thereby will combine to form peroxynitrite.
There is a direct relationship between elevated levels of peroxynitrite, on the one hand, and enhanced NMDA receptor activity (in the later stages of withdrawal) and L-type VGCC activity, on the other, in PWS.	Peroxynitrite and superoxide can cause mitochondrial dysfunction and lead to depletion of ATP. ATP depletion, in turn, can result in partial depolarization of the plasma membrane, which can act to increase activity of both NMDA receptors and VGCC's.
Administration of L-type calcium channel blockers immediately prior to and subsequent to withdrawal reduces formation of peroxynitrite.	Withdrawal potentiates the L-type VGCC's. L-type VGCC blockers inhibit L-type VGCC-mediated Ca^{2+} influx. With less $[Ca^{2+}]i$ available, eNOS and nNOS produce less nitric oxide, and hence, less peroxynitrite will form.
Administration of NMDA receptor antagonists during the later stages of withdrawal lowers formation of peroxynitrite.	NMDA receptor antagonists lower excessive NMDA receptor-mediated Ca^{2+} influx that has been shown to occur in the later stages of withdrawal. With less $[Ca^{2+}]i$ available, eNOS and nNOS produce less nitric oxide, and hence, less peroxynitrite will form.
Supplementation with BH4 immediately prior to and during withdrawal lowers peroxynitrite formation and may be useful in mitigating risk of PWS.	Optimal levels of BH4 lessen the occurrence of partial nitric oxide uncoupling thereby lowering the amount of peroxynitrite formed during withdrawal.
Administration of antioxidants immediately prior to and during withdrawal reduces peroxynitrite formation and may be useful in mitigating risk of PWS.	Antioxidants lower oxidative stress caused by peroxynitrite formed during withdrawal thereby reversing or at least mitigating the NO/ONOO(−) cycle that perpetuates PWS.
Inducing the Nrf2 regulatory system immediately prior to and during withdrawal lowers peroxynitrite levels formed subsequent to withdrawal. Nutritional factors taken immediately prior to and during withdrawal that induce Nrf2 may be useful in mitigating risk of PWS[1].	Inducing Nrf2 activates the antioxidant response elements (ARE's) thereby reducing oxidative stress, an element in the NO/ONOO(−) cycle.

[1] The transcription factor, Nrf2 or nuclear factor erythroid-2-related factor 2, activates the transcription of over 500 genes in the human genome, most of which have cytoprotective effects, including action via over two dozen genes that increase highly coordinated antioxidant activities, and therefore may act to help reverse, or at least prevent, NO/ONOO(−) cycle related illnesses [62]. Nrf2 is known to be activated by various health promoting nutrients [62].

cycle. Additionally, if experimentation provides evidence consistent with this hypothesis, such findings will not only help substantiate PWS as a chronic neuro-dysfunctional illness but will also lend further support for Pall's NO/ONOO(−) theory in general and, perhaps, even augment its ramifications for all implicated illnesses.

Finally, the matter of whether exposure to now ubiquitous microwave radiation associated with cell phone signal transmission and extremely low frequency electromagnetic fields (emf's) produced by wireless internet routers compounds the intensity and prolongs the course of benzodiazepine withdrawal syndrome appears to be worth exploring. In recent years, Pall has focused much of his time researching the extensive body of evidence that shows that low wave emf exposure activates L-type VGCC's and promoting awareness for the potential of such exposure to trigger certain diseases [29,31]. A synergistic pathophysiological effect between benzodiazepine withdrawal and exposure to low wave emf's is plausible because existing evidence suggests that both factors potentiate L-type VGCC's. If experimentation indicates that exposure to low wave emf's intensifies and prolongs withdrawal, then epidemiological studies would be warranted to determine whether PWS has become increasingly prevalent commensurate with the increase in environmental exposure to low wave emf's.

Expected findings in withdrawal from benzodiazepines

Listed in the table below are expected findings from any relevant future research that tests the hypothesis presented in this paper along with rationales.

It is unclear whether withdrawal will result in significant peroxynitrite formation absent low levels of BH4; however, if this is the case, it would suggest that benzodiazepine withdrawal can act as a "pro-oxidant" event independent of partial nitric oxide uncoupling.

Simple blood tests for 3-Nitrotyrosine in humans reportedly afflicted by PWS would reveal whether elevated levels of peroxynitrite are consistently found in such patients. Additionally, behavioral studies could determine whether there is a direct relationship between nitrotyrosine levels and the patient's level of functioning and severity of symptoms. While not dispositive in determining whether PWS is a NO/ONOO(−) disorder, consistent findings of significantly elevated nitrotyrosine in patients is necessary to maintain such possibility.

In addition to confirming the expected findings listed in the table below, research could be conducted to determine (1) protein and cytokine expression in both the early and later stages of withdrawal and (2) which tissues form the most peroxynitrite during withdrawal.

Finally, it is expected that individuals who carry gene variants or polymorphisms that act to lower synthesis or recycling of BH4 and individuals who carry polymorphisms that lower activity of Nrf2 each have an increased risk for developing PWS subsequent to withdrawal from benzodiazepines. Again, lower levels of BH4 result in higher levels of peroxynitrite especially when accompanied by high nitric oxide production, a likely downstream effect of withdrawal. And as discussed in Table 1, if induction of the Nrf2 regulatory system during withdrawal acts to lower peroxynitrite formation via activation of the antioxidant response elements (ARE's), then carrying gene variants that lower the therapeutic activity of the Nrf2 pathway would result in increased risk of PWS.

Conclusion

While risk awareness associated with benzodiazepine discontinuance is generally confined to the acute phase of withdrawal, some experts have observed the propensity of benzodiazepine withdrawal to trigger protracted illness. To date, no one has proposed a

testable mechanism for explaining emergence and propagation for protracted withdrawal symptoms subsequent to discontinuance of chronic benzodiazepine administration. Pall's NO/ONOO(−) theory may apply to PWS because withdrawal appears to be a stressor that substantially raises an element in the NO/ONOO(−) cycle, i.e. L-type VGCC activation and resultant elevated intracellular calcium, and, therefore, may lead to peroxynitrite formation initiating the self-perpetuating NO/ONOO(−) cycle. While the role of L-type VGCC up-regulation to increase glutamatergic strength and reduce $GABA_A$ functioning explains much regarding the pathophysiology in acute withdrawal, NO/ONOO(−) cycle pathophysiology explains how those events in acute withdrawal become chronic.

If experimentation provides evidence to support this theory, then further research should be considered to further explore the epidemiology of PWS, as well as, potential therapies. If PWS is a NO/ONOO(−) disorder, then research of PWS may have ramifications for all NO/ONOO(−) disorders.

Conflict of interest

The author is a co-director of a non-profit organization, Benzodiazepine Information Coalition (BIC) that seeks to raise awareness of the potential for harm of chronic benzodiazepine use. The author does not receive any compensation, monetary or otherwise, for serving in this capacity. Moreover the author has not nor will not receive any compensation, monetary or otherwise, for writing this paper. The purpose of this paper is simply to set forth a testable hypothesis based on the existing body of research of benzodiazepine sensitization and withdrawal.

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