GAD: experimental medicine models, emerging targets

Pharmacotherapy in generalized anxiety disorder: novel experimental medicine models and emerging drug targets

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Accepted for publication in CNS Drugs, 6th March 2017
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Abstract

Many pharmacological and psychological approaches have been found efficacious in patients with generalized anxiety disorder (GAD), but many treatment-seeking patients will not respond and others will relapse despite continuing with interventions which initially had beneficial effects. Other patients will respond, but then stop treatment early because of untoward effects such as sexual dysfunction, drowsiness and weight gain. There is much scope to develop novel approaches, which could have greater overall effectiveness or acceptability than currently available interventions, or which have particular effectiveness in specific clinical sub-groups. 'Experimental medicine' studies in healthy volunteers model disease states and represent a 'proof-of-concept' approach for the development of novel therapeutic interventions: they determine whether to proceed to pivotal efficacy studies, and so can reduce delays in translating innovations into clinical practice.

Investigations in healthy volunteers challenged with the inhalation of air 'enriched' with 7.5% carbon dioxide (CO₂) indicate this technique provides a validated and robust experimental medicine model, mirroring the subjective, autonomic and cognitive features of GAD. The anxiety response during CO₂ challenge probably involves both central noradrenergic neurotransmission and effects on acid-base sensitive receptors, and so may stimulate development of novel agents targeted at central chemosensors. Increasing awareness of the potential role of altered cytokine balance in anxiety and the interplay of cytokines with monoaminergic mechanisms may also encourage the investigation of novel agents with modulating effects on immunological profiles. Although seemingly disparate, these two approaches to treatment development may pivot on a shared mechanism in exerting anxiolytic-like effects through pharmacological effects on acid-sensing ion channels. (253 words)

Key Points

- Generalized anxiety disorder (GAD) is a common and impairing condition for which currently available pharmacological and psychological treatments are not ideal, having sub-optimal efficacy and acceptability problems in both short-term and long-term treatment.

- 'Experimental medicine' studies in healthy volunteers provide useful 'proof-of-concept' approaches in the development of novel pharmacological and psychological interventions: two promising avenues include the development of novel agents targeted at central chemosensors, or at modulating immunological responses.

- Investigations in healthy volunteers challenged with the inhalation of air 'enriched' with 7.5% carbon dioxide (CO₂) indicate this technique provides a validated and robust experimental medicine model, mirroring the subjective, autonomic and cognitive features of GAD.

Compliance with Ethical Standards

Conflicts of interest. Over his academic career DSB has held research grants from the following pharmaceutical and biotechnology companies: Bristol-Myers Squibb, Cephalon, Eli Lilly Ltd, GlaxoSmithKline, H. Lundbeck A/S, Pierre Fabre, Pfizer Ltd, Roche and Vernalis Ltd. He has served on advisory boards hosted by Astra-Zeneca, Bristol-Myers Squibb, Eli Lilly Ltd, GlaxoSmithKline, Grunenthal, H. Lundbeck A/S, Pierre Fabre, and Pfizer Ltd. He is a past President of Depression Alliance and a current Medical Patron of Anxiety UK. RG, NH, RH and MG have no potential conflicts of interest to declare.
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Funding. RG and NH are NIHR Academic Clinical Fellows with supportive grants from the Research Management Committee of the Faculty of Medicine at the University of Southampton. No specific funding was sought or received for the preparation of this review.
1. **Current diagnosis and pharmacological treatment of generalized anxiety disorder**

Generalized anxiety disorder (GAD) is the most common impairing anxiety disorder, having an estimated twelve-month prevalence of 1.7-3.4% (being more prevalent in individuals aged 65 years or more) [1]. Diagnosis currently rests on establishing the presence of psychological and physical anxiety symptoms for at least six months, the symptoms not being understandable as arising from another disorder. In the ICD-10 criteria [2] particular emphasis is given to tension, worry and apprehension, whereas the DSM-5 criteria [3] emphasize multiple and uncontrollable worries. Previous versions of DSM diagnostic criteria (based on the presence of symptoms for one month) had low inter-rater reliability [4, 5], but the requirements of a six-month duration and perception of uncontrollable worry enhanced the reliability of diagnosis [6]. Whilst reliability has increased, some concerns remain about diagnostic validity, including the distinction from depression and the necessary symptom severity threshold [6, 7]. This suggests a more dimensional approach based on worry, distress, and associated symptoms could be beneficial in delineating the condition [8, 9]. In current research settings, symptom severity tends to be assessed through observer-rated scales. Despite inherent limitations including non-specificity for GAD [10] the Hamilton Rating Scale for Anxiety (HAMA) [11] remains widely regarded as the ‘gold standard’: a HAMA score of less than 9 corresponds to symptom remission, a score of 24 or more corresponds to anxiety symptoms of at least moderate intensity [12], and scores are positively correlated with symptom-rated disability [13].

Evidence-based treatment guidelines summarise what is known about the efficacy, tolerability, and clinical roles of currently available pharmacological and psychological treatments. Selective serotonin reuptake inhibitors (SSRI) have become first-line pharmacological treatments in patients with GAD, based on efficacy and tolerability in randomised controlled trials [14]. Despite high placebo response rates, only around one-half of patients exposed to active medication will enter symptom remission at the end of acute treatment [15]. Coexisting depressive symptoms of mild-moderate intensity often diminish with treatment, even with anxiolytic drugs with no proven efficacy in major depression [16, 17]. An early onset of clinical effect (measured by a 20% or greater reduction in symptom severity after two weeks) predicts a greater likelihood of treatment response [18, 19]. There is much uncertainty about the relative efficacy and tolerability of differing pharmacological treatments [20].

As GAD tends to be either episodic in nature or waxing and waning in intensity over many years [21], long-term treatment is usually required. Relapse prevention studies support the long-term efficacy of a range of pharmacological treatments, including some SSRIs (escitalopram, paroxetine) [22, 23], the serotonin-noradrenaline reuptake inhibitors (SNRI) duloxetine and venlafaxine [24, 25, 26], the anxiolytic drug pregabalin [27], the antipsychotic drug quetiapine [28], and the novel antidepressant agomelatine [29].

Despite challenging problems including risks of tolerance and dependence, benzodiazepine anxiolytics are still often employed in routine clinical practice when treating patients with GAD [30]. There is a potential role for judicious prescribing of benzodiazepines in some patients, for example in short-term treatment (up to 4 weeks) whilst waiting for an SSRI to become effective, and in longer-term treatment, when persistently distressed and severely symptomatic patients have not responded to an SSRI, SNRI, pregabalin, the 5-HT \textsubscript{1A} partial agonist buspirone, or to psychological interventions [31]. Some of the pharmacological properties of the ‘multi-modal’ antidepressant vortioxetine and its effects in pre-clinical animal models together suggest potential anxiolytic effects in clinical samples [32]. A meta-analysis of randomised controlled trials (RCTs) found that vortioxetine can reduce anxiety symptoms in depressed patients [33]. A pooled analysis of RCTs in patients with major depressive episodes found no significant difference between vortioxetine and placebo in treatment-emergent anxiety (unlike many antidepressants) [34]. However, placebo-controlled trials of acute
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treatment in patients with GAD have produced inconsistent findings [35], despite clear efficacy in preventing relapse [36]. These differential findings suggest it is possible to delineate more clearly the position of current and potential novel pharmacological treatments in the differing phases of clinical management in GAD.

The sometimes disappointing effects of current medications in relieving anxiety symptoms, and many associated tolerability concerns together provide the imperative to develop novel pharmacological treatments [37]. There is room for development of interventions with an earlier onset of effect (within a few days), greater overall effectiveness, and enhanced effectiveness, whilst avoiding untoward effects such as sedation, weight gain, sexual dysfunction [38], and risks of tolerance and dependence [37]. This article provides a mechanistic rationale for the possible ‘repurposing’ of familiar medications (including amiloride and certain analgesics) as potential anxiolytic treatments and highlights a potentially productive route to novel psychotropic drug development, based on insights from an experimental medicine model of generalised anxiety disorder which involves inhalation of air with an increased proportion of carbon dioxide.

2. Experimental medicine approaches in anxiety disorders

Confirming the potential benefit of novel treatments in the necessary large randomized controlled trials is time-consuming and costly, and novel psychotropic drug development is now often regarded as ‘high risk’, with many biotechnology and pharmaceutical companies reducing investment in neuroscience [39]. There is scope for refining animal models of anxiety disorders and for improving methods for establishing likely anxiolytic properties of novel compounds [40] but successful development of novel anxiolytics may depend upon a refined biomarker approach combining genetic, cognitive and neuroimaging measures [41]. There are typically prolonged delays before empirical innovations translate into clinical practice [42], but ‘experimental medicine’ studies in healthy volunteers can be a useful ‘proof-of-concept’ approach to determine whether there is sufficient encouragement to proceed to necessary pivotal efficacy studies.

Table 1 lists the necessary criteria [43, 44] for an experimental medicine model. A range of such models have been used to support investigation and treatment development in anxiety disorders, including lactate infusion and cholecystokinin challenge in panic disorder, the Trier Social Stress Test in social phobia, oxytocin administration and attachment priming in separation anxiety disorder, and threat of unpredictable shock or carbon dioxide inhalation in panic disorder and GAD. Experimental approaches tend to focus on the physiological, pharmacological or psychological induction of anxiety symptoms [44] and have some limitations: for example, evaluating the effects of novel compounds in drug-induced anxiety can be complicated by drug interactions, and the effects of physiological or psychological challenges are influenced by individual resilience. Anxiety induction following carbon dioxide inhalation is the most comprehensively investigated approach as an experimental medicine model of GAD.

INSERT TABLE 1 ABOUT HERE

2.1. Carbon dioxide (CO2) inhalation

Inhalation of air ‘enriched’ with a higher than normal proportion of carbon dioxide (CO₂) is one of the most frequent experimental approaches in the investigation of induced anxiety, though studies have employed variable procedures, altering the CO₂ concentration, the duration of inhalation, the population sample, and the range of outcome measures. Brief inhalation of air with high concentrations of CO₂ (such as single vital capacity inhalations of 35% CO₂) is associated with the experience of acute severe anxiety, which often includes a panic attack [45]. By contrast, 20-minute inhalation of 7.0-7.5% CO₂ can induce subjective and
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autonomic responses and neurocognitive changes which resemble the features of generalised anxiety: increases in heart rate and systolic blood pressure are observed reliably, but an increase in diastolic blood pressure is less often seen and induction of panic is unusual [44].

The mechanisms underlying the provocation of anxiety by CO₂ challenge are not established fully [46, 47], though genetic factors may be important in CO₂ hypersensitivity [48-49]. Inhalation of air enriched with a high proportion (35%) of CO₂ is associated with increased cortisol secretion [50, 51] but it is unclear whether the cortisol response is specific to CO₂ challenge, or occurs through a more general response to other procedural aspects [44]. The role of disturbed respiratory physiology in panic attack induction following CO₂ inhalation has not been clarified, but experimentally induced panic attacks are associated with low end-tidal CO₂ and high ventilation variance at baseline [52].

Serotonergic mechanisms may influence the panic response to CO₂ challenge. Tryptophan depletion alone does not induce panic [53], but does enhance the panic response to CO₂ inhalation [54], and administration of the serotonin precursor L-5-hydroxytryptophan reduces the panic response [55]. The associations of increased subjective anxiety, heart rate and blood pressure in healthy volunteers after 35% CO₂ challenge also suggest a shared noradrenergic-mediated mechanism underlying CO₂ sensitivity [56]. Changes in CO₂ saturation may act upon pH or CO₂-dependent chemoreceptors within the locus coeruleus (LC) and increase the release of noradrenaline (NA), as 5% CO₂ increases LC neuronal firing rate in rat brain slices [57]. This CO₂-induced NA release may mediate autonomic and subjective features of anxiety through projections to centres involved in cardiovascular control and the limbic system; and the endocrine response may be mediated by altered noradrenergic input into the paraventricular nucleus, enhancing the release of corticotrophin releasing factor and anti-diuretic hormone, so triggering cortisol secretion. Whilst NA is important in mediating anxiety provoked by 35% CO₂ challenge, additional mechanisms must be involved as drugs which affect noradrenergic function have little effect on subjective responses to CO₂ [58].

CO₂ reactivity in mice is linked to chemosensors within the amygdala [59]. The most well-characterized chemosensor is the acid-sensing ion channel 1 (ASIC-1a), a voltage-insensitive H⁺-gated cation channel, highly expressed in the amygdala, dentate gyrus, cortex, striatum and nucleus accumbens [60]. ASICs are pH-sensitive and able to detect small reductions in brain pH (acidosis), such as that arising from inhalation of an acidic gas (including CO₂) [61]. Inhalation of 2-20% CO₂ elicits normal mouse fear behaviour in the presence of fully functioning ASIC-1a chemosensors, but pharmacological blockade or elimination of ASIC1a in knockout mice impairs fear responses to CO₂ whereas subsequent amygdala-localised re-expression restores fear behaviour [63]. Other chemosensor structures include orexin neurones in the hypothalamus, serotonergic neurones in the medullary raphe [63], t-cell death-associated gene-8 receptors in the subfornical organ, and hypoxia-sensitive chemosensory neurones in the periaqueductal gray [46]. Findings in animal models may not translate to humans, and perturbed chemosensor activity may not fully explain the physiological effects of CO₂ challenge which are not understandable solely in terms of CO₂-provoked alterations in noradrenergic activity.

3. **Low-dose CO₂ inhalation as an experimental medicine model of generalized anxiety**

Low-dose (7.5%) but prolonged (20 minutes) CO₂ inhalation was first found to induce anxiety in a double-blind, placebo-controlled trial involving healthy volunteers: when compared to normal (placebo) air inhalation, CO₂ inhalation increased heart rate and blood pressure and heightened subjective anxiety [64]. A subsequent single-blind, placebo-controlled healthy volunteer study found that when compared to air, 7% CO₂ inhalation increased respiratory rate, minute volume and end-tidal CO₂ skin conductance and subjective feelings of anxiety: a sub-group which experienced marked anxiety underwent a second identical inhalation with good
test-retest repeatability. However the study findings highlight potential limitations of the model, as 30% of participants were 'non-responders', and 10% of participants experienced significant anxiety during (placebo) air inhalation [65]. 7.5% CO₂ challenge can reliably induce dysfunction in neuropsychological mechanisms that characterize (unchallenged) trait anxious populations and patients with GAD e.g. hyper-vigilance/alertness [66], poor attention control (increased distractibility) and selective processing of environmental threat [67]. Although inhalation challenges with less than 15% CO₂ provoke significantly more panic attacks in patients with panic disorder than in healthy controls [44], it is uncertain whether altered sensitivity to 'low dose' CO₂ inhalation is also seen in patients with GAD. A small single-blind, randomized, cross-over study which employed a repeated 7.5%, 20-minute inhalation in medication-free GAD patients found that CO₂ inhalation increased subjective anxiety and systolic blood pressure, when compared with air: qualitative assessment of participants' experiences found they resembled their previous symptoms of generalised anxiety, more closely for physiological than cognitive symptoms [68].

4. Effects of current and potential pharmacological treatments on CO₂ inhalation

The effectiveness of psychotropic medication in attenuating CO₂-evoked anxiety has been assessed repeatedly, with variable findings. In general, acute benzodiazepine administration reduces subjective CO₂-provoked anxiety but has little impact on the physiological response. Lorazepam (2 mg) administration attenuated subjective anxiety (with no accompanying change in autonomic measures) when compared to placebo in healthy participants undergoing 20 minute 7.5% CO₂ inhalation [69]. These findings were replicated when lorazepam was employed as a control in studies using the same inhalation procedure to assess novel anxiolytic compounds [70, 71]. Alprazolam (1 mg) and the partial benzodiazepine receptor antagonist zolpidem (5mg) both attenuated subjective anxiety in healthy volunteers after 20 minutes of 7.5% CO₂ inhalation [72]. However a subsequent double-blind, placebo-controlled cross-over study which investigated dose-response relationships with lorazepam - and which used the same experimental paradigm and measures - found no attenuation of subjective or autonomic responses [73].

Administration of various SSRIs, the SNRI venlafaxine, tricyclic antidepressants, or the monoamine oxidase inhibitor toloxatone can attenuate the panic response to CO₂ challenge [44]. As SSRIs often take 4 weeks to exert sustained therapeutic effects in GAD, prolonged drug administration may be needed to generate valid results. A study involving 3 minutes of 5% CO₂ inhalation in individuals 'at high risk of panic disorder' found that 2-week escitalopram administration had no effect on self-report or autonomic indicators of anxiety [74]. By contrast, investigations in patients with panic disorder found that 12 weeks of treatment with different SSRIs or SNRIs reduced subjective anxiety following 5% and 7% CO₂ challenge, when compared to baseline inhalation before treatment [75].

Studies involving SSRI, SNRI or beta-blocker administration in healthy volunteers using a 20-minute 7.5% CO₂ challenge have generated variable findings. As with benzodiazepines, SSRI or SNRI administration has a limited effect on physiological responses to CO₂ challenge. Placebo-controlled administration of the SSRI paroxetine for 21 days (10mg titrated to 20mg after day 8) reduced subjective anxiety [69]. A placebo-controlled investigation of 3-week administration of either venlafaxine (150 mg) or the anxiolytic pregabalin (200 mg) found no significant effect on ratings of subjective anxiety or autonomic response in either the venlafaxine or the pregabalin group [76]. The beta-blocker propranolol (40mg) had no attenuating effect on self-report anxiety in healthy volunteers undergoing 20 minutes of 7.5% CO₂ inhalation [77], which accords with its lack of efficacy in anxiety disorders [78].
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The CO₂ inhalation experimental model may be useful for signalling the potential anxiolytic efficacy of novel therapeutic agents in proof-of-concept studies, including psychological interventions [79]. The model has already been employed in investigations of the CRF₁ receptor antagonist R317573 [70] and the NK₁ receptor antagonists vestipitant and vofopitant [80]. Studies with compounds which target chemosensory mechanisms could inform the development of anxiolytics with a novel mechanism of action. - for example, targeting the ASIC with amiloride, which has been found to have neuroprotective effects [81]. Other potentially fruitful areas include evaluating the effects of orexin receptor antagonists, which can attenuate anxiety-like responses to CO₂ challenge in rats [82]; and targeting the carbonic anhydrase enzyme, responsible for conversion of CO₂ to carbonic acid and thence to hydrogen and bicarbonate ions[46], as acetazolamide (a carbonic anhydrase inhibitor) has been associated with reduced intensity of anxiety and breathlessness during ventilation in patients with panic disorder [83].

5. Cytokines and anxiety disorders

Cytokines are soluble bioactive mediators released by various cell types in the periphery (e.g. monocytes and macrophages) and centrally (e.g. microglia and astrocytes). Cytokine production is dependent upon type 1 helper cells (Th1) which generally mediate a pro-inflammatory cellular immune response, and type 2 helper cells (Th2) which enhance humoral immune reactions. The balance between Th1 and Th2 cytokines is an important determinant of inflammation. Pro-inflammatory cytokines include tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ): they enhance the immune response and speed the elimination of intracellular pathogens. Anti-inflammatory cytokines include interleukin (IL)-4 and IL-10: they enhance phagocytosis of extracellular pathogens, facilitate tissue repair, and attenuate synthesis of pro-inflammatory cytokines [84]. Within the CNS, microglia predominantly secrete Th1 cytokines, and astroglia predominately secrete Th2 cytokines [85, 86].

Cytokine signalling is involved in neurochemical, neuroendocrine, and behavioural processes and a delicate balance of pro- and anti-inflammatory cytokines may be needed for optimal neuropsychological functioning [84, 87-91]. This balance is influenced by the proportions of activated microglia (excess Th1) and astroglia (excess Th2) and by the interplay between activated T cells and CNS glutamate levels [88, 92-94]. Th1-Th2 imbalance can influence tryptophan metabolism by shifting tryptophan catabolism towards kynurenine (instead of towards serotonin) and kynurenine catabolism towards either microglia quinolinic acid (Th1 response-mediated) or astroglial kynurenic acid (Th2 response-mediated) [95, 96]. Early reports of immune disturbance in depressed patients [97, 98] encouraged extensive investigations of disturbances of cytokines in major depression [86]. Current evidence suggests a Th1-predominant immunophenotype that shifts kynurenine catabolism towards microglial quinolinic acid in major depressive disorder, in contrast to a Th2-predominant immunophenotype that shifts kynurenine catabolism towards astroglial kynurenic acid in schizophrenia [86, 88, 94].

An anxiety-specific effect on inflammatory activity in clinically anxious individuals [99] has been described, but there is a need for deeper understanding of the potential role of specific cytokines and immune balance in anxiety states and different anxiety disorders [100]. A meta-analysis of six studies found no overall difference in TNF-α and IL-6 levels between patients with obsessive-compulsive disorder (OCD) and healthy controls [101]. Post-traumatic stress disorder (PTSD) has been associated with increased levels of IL-6, IL-1β, TNF- α, and IFN-γ [102]. Findings in panic disorder have been mixed [103-106] but agoraphobia has been found to be associated with evidence of chronic low-grade systemic inflammation [107]. An investigation of lipopolysaccharide (LPS) - stimulated cytokine profile in patients with OCD or social anxiety disorder suggested that leukocytes of OCD patients (but not socially anxious patients) produced less IL-6 when
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compared with matched controls [108]. The findings of a longitudinal study in GAD patients suggest that observed increased C-reactive protein levels are probably attributable to body mass index and medication use [109]. Another study in GAD patients indicated deficiencies in Th1 and Th2 cytokines following T-cell activation when compared to controls, though Th1:Th2 ratios were not examined [110]. A recent case-control study with comparison of pro-inflammatory to anti-inflammatory cytokine ratios found significantly higher ratios of TNF-α /IL10, TNF-α /IL4, IFN-γ /IL10, and IFN-γ /IL4 in patients with GAD, when compared to matched controls [111].

6. Selective cyclo-oxygenase-2 inhibitors in reducing depressive and anxiety symptoms

Pre-clinical, post-marketing, and treatment studies with traditional non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen have suggested beneficial effects in animal models of depression [112, 113], and ibuprofen had anxiolytic-like effects in an animal model of PTSD [114]. The mechanism underlying potential anxiolytic effects is uncertain, but ibuprofen inhibits both the activity and inflammation-induced expression of acid-sensing ion channels in nociceptors in rodents [115], possibly through decreasing maximal proton-induced current and by slowing down ASIC desensitization [116]. NSAIDs have also been found beneficial in reducing depressive symptoms in patients with osteoarthritis [117], and in enhancing the effects of antidepressants in depressed patients [118]. However not all evidence is supportive [119, 120], and concomitant use of NSAIDs can increase the risk of gastrointestinal adverse effects with SSRI and SNRI antidepressants [121].

Selective cyclo-oxygenase (COX-2) inhibitors (such as celecoxib) are a type of NSAID, and target the enzyme cyclo-oxygenase-2, thereby inhibiting prostaglandin-E2 (PGE2) production and cytokine production: they have a lower risk of associated peptic ulceration than traditional NSAIDs. Placebo-controlled studies have suggested that celecoxib augments the response to antidepressant (reboxetine, fluoxetine) drugs [122, 123], and reduces depressive symptoms when given in monotherapy to patients with physical illnesses [124, 125] (brucellosis, breast cancer). Subsequent meta-analyses provide further support for the beneficial effects of celecoxib in antidepressant augmentation [126, 127].

Additional support for the potential role of COX-2 inhibitors in reducing depressive and anxiety symptoms comes from a series of studies with curcumin, the principal curcuminoid in the spice turmeric, which can down-regulate COX-2 expression and PGE2 synthesis, so representing a ‘natural’ COX-2 inhibitor [128]. Randomized, placebo-controlled antidepressant augmentation studies [129, 130] and randomised placebo-controlled monotherapy studies [131-133] have together shown that curcumin has potential to reduce depressive and/or anxiety symptoms in depressed patients. However it is uncertain whether these effects of curcumin are related to its COX-2 inhibitory properties, or to other pharmacological properties including enhanced monoaminergic neurotransmission, reduced cortisol- and quinolate-induced neurotoxicity, enhanced mitochondrial protection and reduced oxidative and nitrosative damage [128].

7. Conclusions

GAD is a common and impairing medical condition, associated with increased physical and psychological comorbidity, and with increased mortality, some of which may be related to states of chronic peripheral or central inflammation. Current pharmacological and psychological treatments are less than ideal, having sub-optimal short-term and long-term effectiveness and significant acceptability concerns: there is a persistent need to develop new treatment approaches which have enhanced efficacy and improved tolerability.

‘Experimental medicine’ studies performed in healthy volunteers with novel pharmacological compounds or innovative psychological or neuromodulatory interventions can represent a useful ‘proof-of-concept’ approach
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to determine whether pharmaceutical, biotechnology or medical device companies should proceed to pivotal
but more time-consuming and costly formal efficacy studies. The experimental medicine approach may
therefore reduce the typically prolonged delays before treatment innovations are licensed by regulators for
use in clinical samples and adopted into routine clinical practice. For GAD, a body of investigations of the
challenge of healthy volunteers through inhalation of air ‘enriched’ with 7.5% CO₂ suggest this technique
provides a robust experimental medicine model that mirrors the subjective, autonomic and cognitive features
of the clinical condition; and can be used to accompany other experimental models (for example, threat of
unexpected shock, and worry induction procedures) to comprehensively evaluate novel treatment targets.

The anxiety response during CO₂ challenge probably depends upon perturbations in central noradrenergic
neurotransmission and other mechanisms involving acid-base sensitive receptors. Two promising but
seemingly rather disparate pharmacological approaches in the development of novel anxiolytics include the
development of novel agents targeted at central chemosensors (for example drugs with properties similar to
amiloride or acetazolamide) or at modulating immunological mechanisms (for example compounds with
similarities to ibuprofen or celecoxib), though there may be a common mechanism in exerting anxiolytic-like
effects, through shared activities on acid-sensing ion channels [59]. Enhanced awareness of the potential
roles of altered cytokine balance in anxiety, and its potential interplay with monoaminergic and chemosensor
mechanisms, may together encourage the development of novel anxiolytic agents.
Table 1. Requirements for an experimental medicine model in psychiatry

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<tr>
<th>Requirement</th>
<th>Description</th>
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<tr>
<td>safe</td>
<td>for participants and investigators, ideally non-invasive</td>
</tr>
<tr>
<td>acceptable</td>
<td>to participants, ethics committees, regulatory bodies</td>
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<tr>
<td>reliable</td>
<td>inter-performer and repeat-performer replicability</td>
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<tr>
<td>valid</td>
<td>effects attenuated by clinically effective treatments</td>
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<tr>
<td>translational</td>
<td>from lab to clinic and back again, across species</td>
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<tr>
<td>feasible</td>
<td>ease of performance in practice</td>
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<tr>
<td>repeatable</td>
<td>no attenuation of response if performed again</td>
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<td>subjective</td>
<td>measurable psychological effects</td>
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<tr>
<td>objective</td>
<td>measurable physiological effects</td>
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<tr>
<td>inexpensive</td>
<td>can be supported by academia and industry</td>
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For further details see [42] and [43]
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