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## Human pharmacology of current and novel gaba(a)-ergic treatments for anxiety

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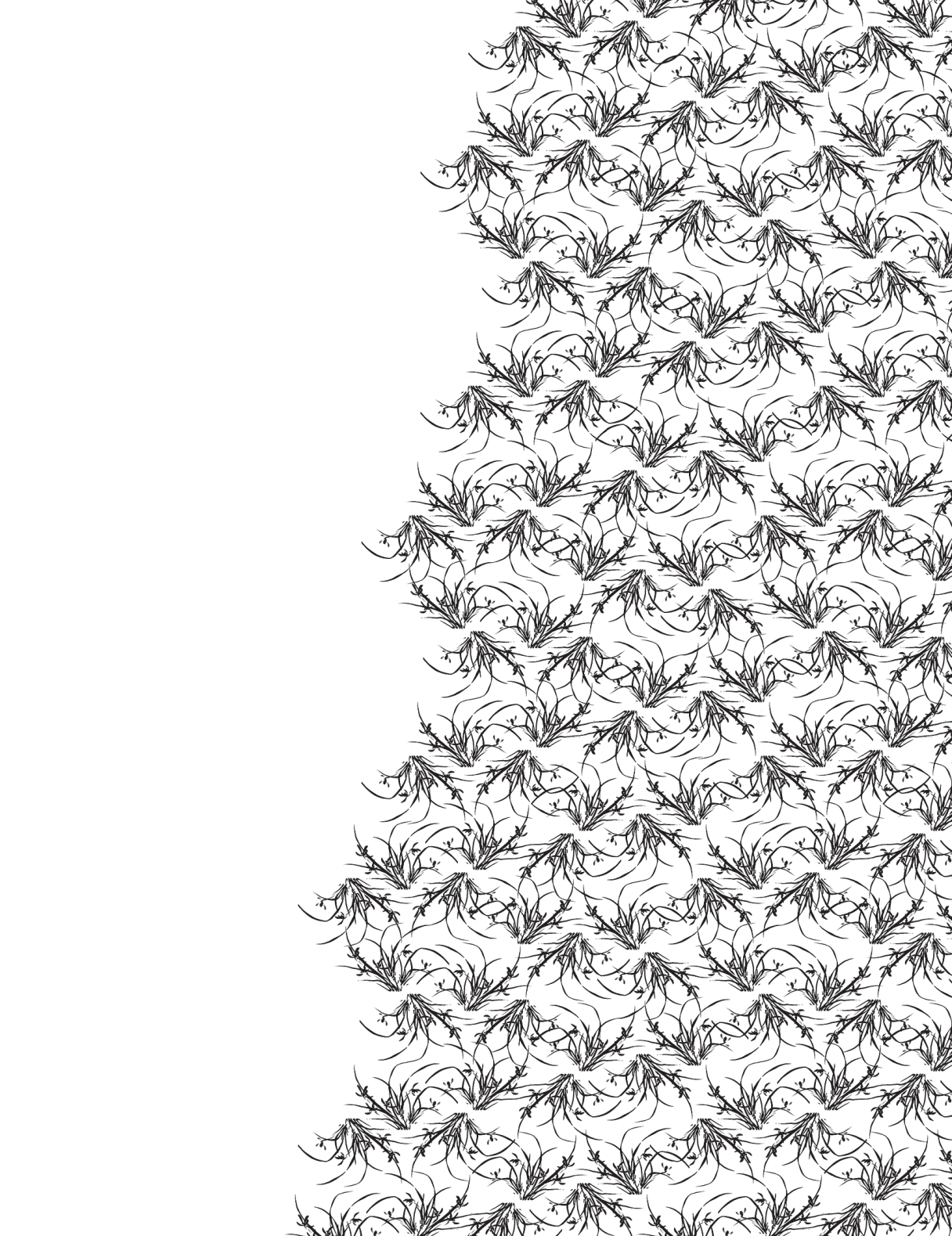
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## **ANXIETY: DEFINITION, DIAGNOSIS, EPIDEMIOLOGY, AND CURRENT TREATMENT STATUS**

Anxiety is a commonly occurring negative human emotional state and is characterized by subjective feelings of worry and fear. By definition, worry or apprehension refers to thoughts and expectations about future events while fear is an acute reaction to perceived imminent danger. Subjective phenomena are usually accompanied by physical symptoms such as increased heart rate, shakiness, fatigue, and muscle tension, as well as cognitive, and behavioral manifestations. Anxiety can be adaptive that occurs in response to a threat and prepares to cope with the environment. However, anxiety becomes pathological when it causes significant personal distress and impairs everyday functioning. In order to be diagnosed with an anxiety disorder, individuals have to experience a certain number of symptoms that are disproportionate to either actual or imagined environmental threat for at least six months [1,2].

Anxiety disorders are chronic, disabling conditions that impose enormous costs both on individuals and on society [3-6]. These disorders are prevalent in Western countries. According to a recent 3-year multi-method study covering 30 European countries, 14% of the total population (i.e., 514 million people) were suffering from anxiety disorders [4]. In the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM 5)* [1], seven anxiety syndromes are classified, including panic disorder, agoraphobia, social anxiety disorder (SAD), generalized anxiety disorder (GAD), specific phobias, separation anxiety disorder and selective mutism. The etiology of anxiety disorders is multifactorial and includes genetic liability to a certain extent for some syndromes. In addition, drug withdrawal, substance/medication (e.g. alcohol, caffeine, and benzodiazepines) abuse and dependence, occupational exposure to organic solvents, and life stresses have been related to the etiology of anxiety disorders while psychiatric complications of endocrine disorders like pheochromocytoma and hyperthyroidism have been demonstrated to mimic anxiety disorders. Taken together, the phenomenologically-based diagnostic classification and the multifactorial nature of anxiety disorders are expected to affect the efficacy of anxiolytic CNS active drugs that have been discovered in the past decades.

Current treatment modalities for anxiety disorders can be categorized into psychological treatments (e.g., exposure therapy, cognitive therapy and cognitive behavioral therapy) and pharmacological interventions [2]. The pharmacological interventions can be further divided to chronic or maintenance treatments and short-term treatments inducing acute anxiolysis. Monoamine modulating drugs such as the selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) are considered the first-line drugs for anxiety

disorders. This is mainly due to their ‘broad spectrum’ anxiolytic efficacy in both short-term and long-term therapy and the relatively good tolerability in terms of side effects and treatment adherence [2]. However, since it is not unusual for treatment response to be reached only after 12 weeks of treatment at a therapeutic dose, the delayed onset of action of SSRIs and SNRIs remains a major disadvantage. In addition, when patients do not respond to or are intolerant of SSRI/SNRI treatment, alternative classes of psychotropic drugs, such as other antidepressant drugs (e.g., tricyclic antidepressants [TCAs], irreversible monoamine oxidase inhibitor [MAOI] phenelzine), anticonvulsant drug pregabalin, antipsychotic drugs (e.g., quetiapine), and anti-histamine drug hydroxyzine, are considered. Nonetheless, even after treatment with multiple anxiolytic drugs, up to 40% of patients with anxiety disorders do not respond to such drugs at all or only respond partially [7]. Given the rapid-onset effectiveness of benzodiazepines (BZDs) in many patients with anxiety disorders, especially in panic disorder, GAD and SAD patients, these drugs are generally reserved for the treatment of patients who have failed to respond to at least three previous treatments (such as after non-response to an SSRI, an SNRI and a psychological intervention). The use of BZDs should however be minimized and preferably be reserved for short-term treatments to mitigate the risks of troublesome sedation, cognitive impairment and discontinuation symptoms after abrupt withdrawal [8] in both short-term and long-term treatment, and to avoid development of tolerance and dependence with prolonged use. Taken together, an obvious unmet clinical need in the pharmacological treatment of anxiety disorders opens an opportunity for novel pharmacological approaches that demonstrate rapid anxiolytic efficacy that is superior to existing treatments and lacks tolerance induction, abuse liability and withdrawal symptoms.

### **THE BRAIN CIRCUITRY INVOLVED IN ANXIETY AND THE ROLE OF GAMMA-AMINOBUTYRIC ACID A (GABA) IN THE AMYGDALA**

On a neurobiological level, anxiety disorders arise from disruption of the highly interconnected circuits normally serving to process the stream of potentially threatening stimuli detected by the human brain from the outside world. Perturbations anywhere in these circuits cause imbalance in the entire system, resulting in a fundamental misinterpretation of neural sensory information as threatening and leading to the inappropriate emotional- and thereby behavioral-responses seen in anxiety disorders [9].

Briefly speaking, anxiety is linked to compromised interactions between the amygdala and the dorsal and ventral medial prefrontal cortex (mPFC). Tract-tracing studies in rats show that axons originating in the infra-limbic cortex of the mPFC terminate most densely in the ventromedial lateral nucleus, the rostral part of the

accessory basal amygdala, lateral capsular subdivision of the central nucleus and the superficial nuclei (lateral olfactory tract, periamygdaloid cortex and cortical nuclei) [10-12]. Neurons in the more caudal areas of the infra-limbic sub-region also project to the medial and intermediate subdivisions of the central nucleus [11,13]. The pre-limbic cortex of the mPFC is located dorsally adjacent to the infra-limbic sub-region and it has a different pattern of connectivity with the amygdala. Pre-limbic cortex neurons target the basal nucleus of the amygdala (BA), primarily the dorso-medial part [11,14], while caudal pre-limbic cortex neurons concentrate inputs in the medial parvocellular basal nucleus [15].

Fear extinction is defined as a decline in conditioned fear responses following repeated exposure to a feared conditioned stimulus (e.g., a tone in both animals and humans) in the absence of the unconditioned stimulus (usually a footshock in animals) with which it was previously paired [16]. Extinct fear can be recovered with time or change of the experimental context, suggesting that fear extinction reflects a learning process. The fear reduction is associated with inhibition rather than erasure of the original fear memory. Given that fear extinction has a close therapeutic analogue in the form of exposure therapy for patients with anxiety disorders, it has been implicated in many preclinical studies to investigate drugs acting as adjuncts to strengthen extinction and reduce intrusive fear memories in PTSD and specific phobias [17]. The acquisition, consolidation and retrieval of extinction therefore are separable processes that are controlled by different brain regions and neural systems [18].

In both experimental animal and human functional imaging studies, the amygdala and the mPFC has been demonstrated to be associated with the regulation of negative emotion, such as anxiety or worry and apprehension. Neuroimaging studies consistently show that higher levels of anxiety are associated with both attenuated ventral medial prefrontal cortex (vmPFC) activity and exaggerated dorsal medial prefrontal cortex (dmPFC) activity [19,20] in the presence of threatening stimuli. In the absence of threatening stimuli (i.e., at rest) Kim and colleagues [21] reported that the negative connectivity normally seen between the amygdala and the dmPFC at rest was attenuated in high anxious subjects, whereas the positive connectivity normally observed between the amygdala and vmPFC at rest, manifested as negative connectivity in high anxious subjects. Interestingly, the mPFC-amygdala coupling is inversely correlated with self-reported measures of anxiety or anxious temperament, indicating that the mPFC functions to actively regulate the amygdala and impaired connection between the two neural structures may lead to inadequate response to threatening stimuli. On the other hand, the amygdala – nuclei situated in the median temporal lobes – appears to play a crucial role in the regulation of negative affect and therefore anxiety-related symptomatology.

Emerging evidence from functional magnetic resonance imaging supports that amygdala is the key brain region of activity in response to negative emotional stimuli in healthy volunteers [22-24]. Besides, patients with anxiety disorders are prone to amygdala activation in response to a given threatening stimulus more than the non-anxious controls [25]. Moreover, successful treatment of anxiety disorders with cognitive behavioral therapy leads to extinction of this hyperactivation in the amygdala [26]. Taken together, mPFC functions to regulate amygdala function by actively suppressing activity, and so deficiency in the top-down regulation of mPFC and hyperactivation of the amygdala have been implicated in the pathophysiology of anxiety-related disorders.

In the amygdala, two groups of nuclei should be noted, namely the basolateral amygdala complex (BLA) and the centromedial amygdala complex, in particular the central nucleus (CeA) [27,28]. The BLA receives afferent information on potentially negative emotional signals from the thalamus and the sensory association cortex. The BLA activates the CeA either directly through an excitatory glutamatergic pathway or indirectly by activating a relay of inhibitory GABAergic interneurons that lie between the BLA and the CeA and exert an inhibitory influence upon the latter [29,30]. The CeA is the principal efferent pathway from the amygdala. Inhibitory GABAergic neurons project from the CeA to the hypothalamus and brainstem; the activation of these neurons leads to the somatic manifestations of anxiety [31]. Projections to other basal forebrain nuclei such as the ventro tegmental area and the locus ceruleus may be involved in the subjective effects that are related to anxiety, such as apprehension and dysphoria [32]. In addition, neurons from the BLA also activate cells in the adjacent bed nucleus of the stria terminalis, which project to the same areas as the CeA and apparently play a similar role [28,32].

The knowledge about the neurobiology underlining anxiety disorders serves as the basis for the search of novel anxiolytic agents. Compounds that manipulate this potential pathway may provide new options for the treatment of anxiety disorders. Moreover, neuroimaging and neurophysiological measurements that address the corresponding processes may be used to assess human responses to drug-mediated target modulation.

### **THE INVOLVEMENT OF GABA SYSTEM IN THE PATHOPHYSIOLOGY OF ANXIETY AND ANXIETY DISORDERS**

Mounting evidence has suggested the pathogenesis of human anxiety disorders is related to a dysfunction of central top-down inhibitory mechanisms. By providing the major source of inhibitory neurotransmission in the mPFC and amygdala, GABA exerts a powerful influence on a range of fear- and anxiety-related behaviours,

including fear extinction [33-37]. Temporary inactivation induced by GABA(A) receptor agonists has been implicated to establish necessary contribution of the infralimbic subregion or basolateral amygdala (BLA) (but not prelimbic cortex) to fear extinction [38,39]. Infusions of GABA or GABA receptor agonists into the amygdala were found reducing measures of fear and anxiety (possibly related to effects on memory reconsolidation) in several animal species [40,41]. On the other hand, infusion of the GABA antagonist bicuculline was found to block chlordiazepoxide-induced anxiolytic-like activity in rats, whereas injecting bicuculline methiodide to the anterior basolateral amygdala of rats elicited anxiogenic-like effects in both the social interaction paradigm and the conflict paradigm. Microinjection of bicuculline methiodide into the central nucleus of the amygdala elicited no change in experimental anxiety [42].

In humans, administration of benzodiazepines is translated to anxiolytic effect by attenuating amygdala activation in response to negative emotional stimuli [43,44]. To the contrary, Nutt et al. [45] performed an interesting study, in which they injected the benzodiazepine-antagonist flumazenil to 10 patients with panic disorder and 10 control subjects. Subjective anxiety responses after flumazenil infusion were significantly higher in patients with panic disorder than in the controls, and panic attacks were successfully induced in eight patients with panic disorder but no panic attack occurred in the controls. Although such findings have not been replicated [46], they are regarded as a potential signal for the possible shift of the “receptor set-point” [45]. Nikolaus et al reviewed 14 nuclear neuroimaging (Positron emission tomography [PET] and Single-Photon Emission Computed Tomography [SPECT]) studies conducted in patients with anxiety disorders (160 patients [mostly GAD patients] vs. 172 healthy controls). They identified a widespread decline of GABA(A) receptor binding sites and reduced binding extent in the whole mesolimbocortical system in patients suffering from anxiety disorders, suggesting attenuation of physiological central depression. The disturbances of the downstream dopaminergic and serotonergic neurotransmission are thought to, at least partly, result from the diminished tone of GABAergic neurotransmission [47]. A decrease of cortical GABA neurons and reduction of GABA levels were reported in patients with major depressive disorder (MDD) using proton magnetic resonance spectroscopy [48]. Considering the frequent comorbidity of MDD with anxiety states, a shared underlying pathology that emphasizes the causal contribution of GABAergic deficit is proposed for both anxiety disorders and depression [49-51]. Similar GABA(A) receptors reduction is also seen in patients with panic anxiety or post-trauma stress disorder (PTSD). Noteworthy, the extent of GABA(A) receptor deficit is significantly correlated to the clinical severity of these two disorders [52-56], suggesting an ‘exposure’-response relationship and hence reinforcing the contribution of GABAergic deficit to anxiety status.



In summary, all aforementioned research findings suggest GABAergic neurotransmission in the mPFC-amygdala coupling is a promising target for modulation of anxiety-related responses.

### **GABA(A) RECEPTOR STRUCTURE, FUNCTION, AND ITS IMPLICATION IN THE PHARMACOTHERAPY OF ANXIETY DISORDERS**

The discovery of the GABA(A) receptor in the 1970s, originally called benzodiazepine receptor, was essential for elaborating the mechanism of action of benzodiazepines, it was the recognition of benzodiazepine-sensitive GABA(A) receptor subtypes that opened up a new GABA pharmacology [57].

GABA(A) receptors belong to the class of ligand-gated ion channels [58]. The GABA(A) receptors are hetero-pentamers traversing the neuronal membrane. To date, a large number of GABA(A) receptor subtypes have been identified:  $\alpha$  1-6,  $\beta$  1-3,  $\gamma$  1-3,  $\Delta$ ,  $\delta$  1-3,  $\theta$ ,  $\pi$  [59]. The majority of GABA(A) receptors in the brain are comprised of two  $\alpha$  subunits, two  $\beta$  subunits, and a  $\gamma$  sub-unit. These subunits construct a cylinder. Activation of the receptor by GABA leads to a conformational change in the protein subunits and results in transient opening of a pore along the axis of the cylinder, allowing the flow of chloride ions from one side of the membrane to another [60]. The pharmacological interaction between benzodiazepines and GABA(A) receptors occurs at a different site independent from the GABA binding site on the GABA(A) receptor. GABA binds within the two interfaces between the  $\alpha$  and  $\beta$  subunits on the GABA(A) receptor. Benzodiazepines bind within the interface between the  $\alpha$  and  $\gamma$  sub-units, thereby potentiating GABA-related activation of the chloride conductance through allosteric modulation [61]. Nevertheless, such benzodiazepine recognition site does not exist in all  $\alpha$  and  $\gamma$ 2 subunit combinations. Therefore, although GABA(A) receptors containing  $\beta$ ,  $\gamma$ 2 plus either  $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 3, or  $\alpha$ 5 subunits possess a binding site for classical benzodiazepines, analogous receptors containing  $\alpha$ 4 or  $\alpha$ 6 subunits do not. The research by Seeburg et al has attributed the benzodiazepine-sensitivity of  $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 3, and  $\alpha$ 5 subunits to the histidine residue in a homologous position in their N-terminal extracellular region, which switches to an arginine residue in the benzodiazepine-insensitive  $\alpha$ 4 and  $\alpha$ 6 subunits [62].

Given the evolutionary preservation of the GABA(A)/Gly receptor-like (GRL) gene sequences in the vertebrates [63], the function of each GABA(A) receptor subunit was initially investigated through a gene knock-out approach. Thanks to the gained experience in gene targeting techniques that enables introduction of specific point mutations, and the recognition that a single amino acid residue in the  $\alpha$  subunit determines the sensitivity of a GABA(A) receptor to diazepam, point mutation of the histidine to an arginine in the  $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 3, and  $\alpha$ 5 subunits was employed in *in vivo* animal studies to convey the interaction between benzodiazepines and the

$\alpha_{1,2,3,5}$ -containing GABA(A) receptors from agonism to inverse agonism [64]. This knock-in approach was used to investigate the underlying pharmacological action of the manipulated receptor subunit.

Based on various experimental knock-in and knock-out mice models,  $\alpha_1$ -containing GABA(A) receptors are linked to sedative effect [65-68], while spinal  $\alpha_2/\alpha_3$  GABA(A) receptors are found to mediate analgesia [69-71] and  $\alpha_5$ -containing GABA(A) receptors, which relatively specifically express in the hippocampus (the central domain for learning and memory), are associated with cognition [72-77]. The GABA(A) subtype responsible for the anxiolytic effects of benzodiazepines are less clear. The involvement of  $\alpha_2$  GABA(A) receptors in anxiolysis is anticipated given their high expression in human amygdala-prefrontal circuitry [78,79]. Most studies suggest that the  $\alpha_2$  rather than the  $\alpha_3$  subtype is related to the benzodiazepines-induced anxiolysis [80,81], while pharmacological studies using either an  $\alpha_3$ -selective inverse agonist [82] or an  $\alpha_3$ -selective agonist [83] implicates the  $\alpha_3$  subtype. Despite of the controversies, the affinity and efficacy of current investigational compounds acting at the  $\alpha_2$  and  $\alpha_3$  subtypes are mostly similar at the  $\alpha_2$ - and  $\alpha_3$ - subunits containing GABA(A) receptors [84].

#### **NOVEL $\alpha_{2,3}$ -SUBTYPE SELECTIVE COMPOUNDS FOR ANXIOLYSIS**

In contrast to other areas of pharmacology, in the field of GABAergic receptor modulator, it has been particularly difficult for medicinal chemists to develop subtype-selective ligands [85], mainly because the high flexibility of GABA(A) receptors and the existence of multiple drug-binding sites. In addition, the distinct subunit composition among the GABA(A) receptor subtypes, the contribution of distinct subunit sequences to binding sites of different receptor subtypes, as well as the fact that even subunits not directly connected to a binding site are able to influence affinity and efficacy of drugs, contribute to a unique pharmacology of each GABA(A) receptor subtype [86].

The binding and efficacy profiles of candidate  $\alpha_{2,3}$  subtype-selective drugs can be classified to either binding-selectivity or efficacy-selectivity. A compound with binding-selectivity is expected to have higher affinity for  $\alpha_2$  and/or  $\alpha_3$  subtypes in vitro and hence specific receptor occupancy and CNS distribution in vivo. Even though the compound may have comparable efficacy at the four benzodiazepine-sensitive GABA(A) receptor subtypes, its pharmacological selectivity is determined in vivo by preferential occupancy. As for efficacy-selectivity, an ideal compound should have opposite pharmacological interactions at different subtypes. In other words, it should exert agonism at the  $\alpha_{2,3}$  subtypes whereas present antagonism or inverse agonism at the  $\alpha_1$  and  $\alpha_5$  subtypes. Between these two

extreme conditions, there could be multiple permutations, including a compound behaves as a full agonist or a relatively high partial agonist at  $\alpha_2$  and/or  $\alpha_3$  subtypes but has weak or none activity at the  $\alpha_1$  and  $\alpha_5$  subtypes.

Based on these principles, a number of conceptually GABA(A)  $\alpha_{2,3}$  subtype-selective compounds have been identified through *in vitro* studies using recombinant human GABA(A) receptors and carried forward into clinical development. Because of their pharmacological selectivity, these compounds are expected to have favorable therapeutic effect with less sedating or cognition impairing effect. Table 1 listed the *in vitro* pharmacological properties of these novel GABAergic compounds.

**Table 1 • In vitro pharmacological properties of the GABAergic compounds**

Compound	$\alpha_1$		$\alpha_2$		$\alpha_3$		$\alpha_5$	
	K <sub>i</sub> <sup>1</sup> (nM)	Efficacy <sup>2</sup> (%)	K <sub>i</sub> (nM)	Efficacy (%)	K <sub>i</sub> (nM)	Efficacy (%)	K <sub>i</sub> (nM)	Efficacy (%)
TPA023 <sup>3</sup>	0.27	0#	0.31	11	0.19	21	0.41	5
MK-0343 <sup>3</sup>	0.22	18	0.40	23	0.21	45	0.23	18
SL65.1498 <sup>4</sup>	17	45	73	115	80	83	215	48
Zolpidem	20	75 <sup>5</sup>	400 (d)	78 <sup>5</sup>	400 (d)	80 <sup>5</sup>	5000(d)	9 <sup>5</sup>
AZD7325 <sup>6</sup>	0.5	0	0.3	18	1.3	15	230	8
AZD6280 <sup>7</sup>	0.5	0	21	32	31	34	1680	7
NS11821 <sup>8</sup>	1.6	4	9.7	17	3.8	40	2.5	41

1. K<sub>i</sub> = constant of receptor-subtype binding / 2. Relative efficacy is defined as the extent of the potentiation of GABA(A) EC<sub>20</sub>-equivalent current produced by the compound compared to that produced by a nonselective full agonist (chlordiazepoxide/diazepam) / 3. Mean values of 3 experiments in *Xenopus* oocytes with human recombinant  $\alpha\beta\gamma_2$  receptors; efficacy relative to chlordiazepoxide [86,89] / 4. Mean values of 3 experiments in HEK293 cells with recombinant rat receptors  $\alpha\beta\gamma_2$ ; efficacy relative to chlordiazepoxide [97] / 5. Mean values of 3 experiments in *Xenopus* oocytes with human recombinant  $\alpha\beta\gamma_2$  receptor; efficacy relative to diazepam [98,99] / 6. Data adapted from [100] / 7. Data adapted from [101] / 8. Data adapted from [102].

## EVALUATION OF HUMAN PHARMACOLOGY

BZDs exert their CNS actions in a concentration-dependent manner [87]. The anxiolytic, hypnotic, muscle relaxant, and amnesic effects of benzodiazepines generally appear concomitantly, and the onset and duration of action correlate closely with the pharmacokinetic profiles of these compounds. Based on non-clinical investigations using *in vitro* assays and animal models of anxiety, the human pharmacology of novel GABAergic agents is approached through clinical pharmacology studies investigating pharmacokinetics, receptor occupancy, and pharmacodynamics (PD) in healthy volunteers. Direct links have been proposed between plasma drug concentration and GABA receptor occupancy [84], as well as between plasma drug concentration and the pharmacodynamic measurements

[88-91]. Such pharmacokinetic/pharmacodynamic (PK/PD) relationships warrant the use of surrogate biomarkers in healthy volunteers treated with single-dose administration of selective novel GABAergic compound(s).

More than 170 pharmacodynamic tests or test variants have been developed to assess the CNS effects of benzodiazepines. De Visser et al. [87] analyzed the inter-study consistency, sensitivity, and pharmacological specificity of the frequently used biomarkers. Saccadic peak velocity (SPV) and visual analogue scale of alertness (VAS<sub>alertness</sub>) were identified as the most sensitive parameters for benzodiazepines. Both measurements showed consistently dose-dependent responses to a variety of benzodiazepines. Based on these findings, the Centre for Human Drug Research (CHDR) has established a selection of computerized CNS-pharmacodynamic tests called the Neurocart battery [92]. The components of this battery target a variety of neurophysiological and/or neuropsychological domains (Table 2).

**Table 2 - Component tests of the Neurocart battery and the related CNS domains**

Neurocart test	Targeted function	Related CNS domains
Saccadic eye movement	Neurophysiologic function	Superior colliculus, substantia nigra, amygdala
Smooth pursuit	Neurophysiologic function	Midbrain
Adaptive tracking	Visuo-motor coordination	Neocortex, basal nuclei, brain stem, cerebellum
Body sway	Balance	Cerebellum, brain stem
Visual verbal learning test (VVLT)	Memory	Hippocampus
VAS Bond and Lader	Alertness, mood, calmness	Cortex, prefrontal cortex
VAS Bowdle	Feeling high, internal and external perception	Cortex, prefrontal cortex, amygdala

Of this battery, adaptive tracking, saccadic eye movements, and body sway were proven sensitive to the sedating effects of sleep deprivation [93], as well as to the effects of benzodiazepines and other GABAergic hypnotic drugs [89,91]. In the recent years, the Neurocart battery was used in a series of phase I studies to assess CNS pharmacodynamics of partial  $\alpha_{2,3}$  subtype selective GABA(A) agonists. Both nonselective and/or selective GABA(A) agonists were administered as single oral dose to healthy volunteers. Clear distinctions were observed between the effect profile of non-subtype-selective full GABA(A) agonist and that of selective partial GABA(A) agonist in these trials [88-90], probably because the subtype specificity of the pharmacodynamic measurements for the pharmacological modulation of GABA(A)-ergic compounds. Unfortunately, none of the novel receptor subtype-selective compounds have reached the market: the development of GABA(A) receptor  $\alpha_2$  and  $\alpha_3$  subunit agonist SL65.1498 [90], was discontinued owing to unexpected amnestic effects, while the phase 2 studies of another compound of this drug class, TPAO23, were terminated prematurely due to preclinical toxicity (cataract

formation) in long-term dosing studies [94], despite exhibiting anxiolytic activity in GAD; MK-0343 also displayed an anxiolytic profile in animal models but produced sedation in humans at low levels of receptor occupancy (<10%) [95].

In summary, these reports indicate that the human pharmacodynamic approach with sensitive and CNS-domain specific neuropsychological and neurophysiological measures is useful in predicting the drug's clinical effect on the central nervous system. Inter-species difference is also noted between human and rodents or primates: although a low *in vitro* efficacy at the  $\alpha_1$ -containing GABA(A) receptors may not lead to an overtly sedative effect in the experimental animals, it apparently causes sedation in humans at comparable exposure levels. The following questions remain to be answered: 1) is reduction of saccadic peak velocity a promising surrogate marker for clinical anxiolysis? 2) can we also differentiate partial agonism from the full agonism of benzodiazepines via this pharmacodynamic package? 3) is such selective CNS-pharmacodynamic effect profile characteristic for the family of GABA(A)  $\alpha_{2,3}$ -subtype receptor agonists?

## CONCLUSION AND AIM OF THESIS

Anxiety disorders are highly prevalent psychiatric disorders and have high personal and societal costs. The transition from “normal” negative affect or anxiety to an anxiety disorder is implemented by the interplay between psychosocial stressors and a wide array of neurobiological alterations which lead to subjective suffering and functional impairment. Monoamine modulating treatments are widely applied to treat anxiety disorders but are not effective in a large proportion of patients. As the predominant inhibitory neurotransmitter system in the human brain, the GABAergic system in general and its  $\alpha_{2,3}$  subunit-containing GABA(A) receptor subtypes in particular, have been implicated in the pathophysiology of anxiety disorders. Novel pharmacological treatments selectively targeting the anxiolysis-mediating GABA(A) receptor subtypes are currently emerging. These range from affinity-selective agents to efficacy-selective agents and represent potentially useful future pharmacological treatments for anxiety disorders [95].

In this thesis, we report several human pharmacology studies that were performed to identify the pharmacologically active doses/exposure levels of several novel compounds with potential anxiolytic effects (Chapter 2, 3, 4). Because of their pharmacological selectivity at the  $\alpha_{2,3}$  GABA(A) receptor subtypes, the novel drugs were expected to elicit clinical anxiolysis and less sedating effects. An overview of the performance of the selected and validated pharmacodynamic measurements is composed to summarize the utility of these neurophysiological and neuropsychological biomarkers in early clinical development of novel anxiolytic drugs

(Chapter 5). However, the difficulty of evaluating therapeutic anxiolytic drug effects in healthy volunteers has led to further explorations on the neuroendocrine biomarkers (Chapter 6) and the integration of a stress-challenging procedure into the evaluations (Chapter 7).

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