

Modelling Anxiety in Humans for Drug Development

Martin Siepmann^{1,*} and Peter Joraschky²

¹*Institute of Clinical Pharmacology and* ²*Clinic for Psychotherapy and Psychosomatic Medicine, Medical Faculty, Technical University, Dresden, Germany*

Abstract: Animal behavioural profiles are commonly employed to investigate new therapeutic agents to treat anxiety disorders as well as to investigate the mechanism of action of anxiolytic drugs. However, many clinically important symptoms of anxiety can not be modelled directly in animals. Human models of anxiety should bridge between animal models and anxiety disorders. Experimental anxiety states in humans can be induced by either pharmacological means such as CO₂ inhalation or psychological means such as aversive conditioning of skin conductance responses to tones. Investigation of these models may contribute to a better understanding of anxiety disorders, both from a biological and behavioural point of view. In a comprehensive review existing models of human experimental anxiety states are summarized and validity is discussed.

Key Words: Anxiety, model, anxiolytic, drug development, humans.

INTRODUCTION

Anxiety is an alerting signal. It warns of impending danger and enables a person to take measures to deal with internal or external threat. The experience of anxiety comprises the awareness of autonomic and motoric reactions such as sweating and shaking as well as the awareness of being frightened. In addition to autonomic and motor effects anxiety impairs cognitive functions. It may produce confusion, distort perception and interfere with learning. Pathologic anxiety is characterized by an overestimation of danger in a given situation and an underestimation of ability to cope with a perceived threat.

Anxiety disorders are extremely common and debilitating multifactorial conditions, and without knowledge of both clinical and biochemical aspects of the diseases it is impossible to develop effective treatment strategies for patients.

Animal behavioural profiles such as locomotion, self-grooming, defecation and urination have long been used to detect effects on anxiety [79] and a number of models, based on animal emotional reactivity, have been proven to be sensitive to stressful manipulations [35]. Animal models of anxiety evaluate conditioned and unconditioned behaviour [74]. Many of these models have been successfully used to test new anxiolytic drugs by simple, rapid and inexpensive ways of evaluating animals' conditions. But is animal anxiety a good approximation of human disorder? There are several limitations. First, many clinically important, especially cognitive-based, symptoms of anxiety cannot be directly modelled in animals. Second, behavioural measures are often confounded and reflect changes in general activity [8]. Third, often poor correlation is noted between different behavioural measures taken in the same test, or the same measures taken in different tests [65]. For example, self-grooming and defe-

cation can often be seen as the only behaviours that change in tests designed to measure anxiety. Due to ethical and economical constraints experiments that induce anxiety states in humans are less frequently used. Nevertheless, they may constitute a helpful bridge between animal models and clinical disorders. Experimental anxiety in humans may be induced by either chemical (i. e. CO₂ inhalation) or psychological means. In this class of tests environmental stimuli or contexts are used to induce anxiety states in humans. However, there are some difficulties in evoking anxiety that is sensitive to anxiolytic drugs because of the low level of anxiety that is allowed by ethical constraints. Further, the sedative and anxiolytic effects of drugs are often difficult to differentiate. Despite of these limitations, some procedures have yielded valuable results. The principal tests used are discussed in the following review.

USE OF CHEMICAL MEANS

Carbon Dioxide

Interest in CO₂ as a model of experimental anxiety has focused on the inhalation of low concentrations (5-7 % CO₂) over 15-20 minutes [41], or a high concentration (35 %) delivered as a single vital capacity inhalation [109]. Both models are well validated and seem to be reliable in evoking anxiety symptoms in patients with panic disorder, but not in healthy controls and neither in patients with obsessive compulsive disorder nor in those with mood disorders [43, 80, 81]. CO₂ reactivity is found attenuated in patients with panic disorder by pre-treatment with benzodiazepines, MAO-A-inhibitors and SSRIs [83, 84, 93]. It has been hypothesized that CO₂ induces anxiety by stimulation of respiratory centres, which are hypersensitive in patients with panic disorder and in their first-degree relatives. Therefore, hypersensitivity to CO₂ has been suggested as a trait marker of the disease [82]. However, one cannot regard CO₂-induced anxiety as identical to natural panic, and we do not know how valid a laboratory model for panic it provides. Among other problems the CO₂ challenge lacks specificity due to a strong influence of cognitive and emotional states on the likelihood that CO₂ will induce panic [92].

*Address correspondence to this author at Institute of Clinical Pharmacology, Medical Faculty, Technical University, Fiedlerstr. 27, 01307 Dresden, Germany, Fax: +49-351-4584341; E-mail: martin.siepmann@mailbox.tu-dresden.de

It has been proposed by Klein and Gorman that CO₂ stimulates the locus coeruleus, an area with a high concentration of noradrenergic cell bodies [66]. CO₂ may thus induce anxiety by stimulating central norepinephrine release. The link between anxiogenic action of CO₂ and sympathetic activation is interesting, but it has yet not been established that panic induced by CO₂ is associated with increased catecholamines [112]. Hypothalamic-pituitary-adrenal (HPA) axis is another main stress response system. Increases in plasma cortisol and ACTH levels were found following inhalation of 35 % CO₂ in healthy subjects [106]. However, it is unclear whether or not panic symptoms are accompanied by activation of the hypothalamic-pituitary-adrenal (HPA) axis [7].

Sodium Lactate

Intravenous infusion of sodium lactate 0.5–1M may induce panic in patients with panic disorder. Across 13 studies reviewed by Markgraf and colleagues [72] 56 % of the patients (110 of 197) panicked after roughly 12 minutes of lactate infusion whereas only 9 % of the healthy control subjects (7 of 76) experienced anxiety, usually after a longer period of time (15–18 minutes). Patients with generalized anxiety disorder seem to respond to a similar extent as patients with panic disorder when being challenged with sodium lactate [68]. By contrast, patients with social phobia do not appear to panic to lactate as suggested by a study conducted by Liebowitz and colleagues [71]. Anxiety states induced by sodium lactate can be attenuated by benzodiazepines and tricyclic antidepressants [70, 86] whereas beta-blockers are not effective [42]. As is the case with CO₂ there is a strong influence of cognitive and emotional states on the likelihood that lactate challenge will induce panic, and baseline differences expressing anticipatory anxiety predict the results of an infusion with sodium lactate [88]. The mechanism that underlies panic attack induced by lactate has yet not been clarified. Pitts and McClure originally proposed a causal link between elevated serum lactate and pathologic anxiety [85]. However, empirical findings made it clear that elevated serum lactate was neither necessary nor a sufficient condition for pathologic anxiety [1, 54]. A number of other biological mechanisms have been discussed as explanation for panic caused by lactate-infusions [37, 40, 44]. But, it has been impossible to show that single mechanisms are necessary or sufficient for panic. Lactate-induced panic can occur in humans without peripheral catecholamine surges, hyperventilation, or hypoglycaemia; thus, these mechanisms are not necessary causes. Hypocalcaemia and alkalosis may occur in persons who do not panic, thus these mechanisms are not sufficient causes. Intravenous lactate infusion was found to increase brain and cerebrospinal fluid lactate levels both in patients with panic disorder and in healthy controls in a series of magnetic resonance spectroscopy studies conducted by Dager's group [30–32, 69]. In these studies panic-disorder patients had greater brain lactate increases in response to lactate infusions as compared to normals. But, severity of symptoms provoked by lactate infusion did not correlate with brain lactate response.

Cholecystokinin

Cholecystokinin (CCK) is a family of peptides, which have long been known to exist in the gastrointestinal tract of

mammals. It was first reported by Vanderhaeghen and colleagues that CCK molecules exist in the CNS of mammals [105]. Evidence followed that some CCK fragments, especially the sulphated octapeptide form (CCK_{8S}), act as neurotransmitters [6]. Interest in the role of CCK in anxiety stems from microiontophoretic studies by Bradwejn and de Montigny on the effect of benzodiazepines on CCK_{8S}-induced excitation of rat hippocampal pyramidal neurons [13]. Despite the evidence that CCK_{8S} does not readily cross the blood brain barrier [114], electrophysiological studies have reported that the intravenous administration of this peptide can produce activation of CNS neurons [36, 61]. This suggests that even minute amounts of the peptide might be sufficient to modify CNS neuronal firing activity. CCK₄, the tetra peptide form of cholecystokinin, was chosen for use in human studies as the smaller size of CCK₄ might allow a greater proportion of the amount injected intravenously to cross the blood-brain barrier. Rehfeld who injected CCK₄ to himself and to healthy volunteers reported symptoms of anxiety, choking and unreality following administration of the substance [89]. In a pilot study, the behavioural effects of CCK₄ were evaluated in patients with panic disorder, and it was hypothesized that CCK₄ could induce panic attacks that are very similar or identical to spontaneous panic attacks [17]. A further study conducted by Bradwejn supports that patients equate panic attacks induced by CCK₄ to their usual attacks in the type and quality of symptoms [18]. Patients with panic disorder show an enhanced sensitivity to the anxiogenic effects of CCK₄ when compared to healthy controls or their first-degree relatives [19]. CCK₄-induced anxiety can be blunted by pre-treatment with antidepressants such as imipramine, fluvoxamine, citalopram and GABAergic anxiolytics such as lorazepam, vigabatrin and tiagabine [14, 94, 107, 116].

Any number of neurotransmitter systems could be mediators of the effects of CCK₄ in humans. The first neurotransmitter system, which deserved study, was the CCK system. Two main types of CCK receptors have been described [34]: CCK_A receptors which have a higher affinity for CCK_{8S} and gastrin and CCK_B receptors, which have a high affinity for all of these compounds. CCK antagonists with selectivity for CCK receptor subtypes have been developed. It was demonstrated in a study with panic disorder patients that L-365, 260, a selective CCK_B receptor antagonist, can block CCK₄-induced panic attacks [16]. A mediating role of the CCK_B system in CCK₄-induced panic attacks has therefore been suggested. Animal studies support this hypothesis: pre-treatment with CCK_B antagonists markedly reduces anxiety provoked by CCK receptor agonists in rodents [58]. The second system studied was the benzodiazepine receptor. Benzodiazepine receptor agonists, by their action on benzodiazepine receptors, exert anxiolytic effects; CCK₄ through actions on CCK_B receptors may act on benzodiazepine receptors in an opposite manner and elicit panic symptoms. If this hypothesis came true, one may expect that a benzodiazepine receptor antagonist would antagonize the panicogenic effects of CCK₄. In a study in healthy subjects the benzodiazepine receptor antagonist flumazenil did not have any effects on number, intensity and duration of symptoms following challenge with CCK₄ [15]. These findings suggest that benzodiazepine receptors are not mediators of the panicogenic action

of CCK₄ in healthy subjects. However, these findings should not be generalized to patients with panic disorder since it is still possible that CCK₄ may act through benzodiazepine receptors in promoting panic attacks in patients. It has been assumed that the biological support for the panicogenic effect of CCK₄ is based on several neurotransmitter systems interacting in a complex, dynamic process. Unfortunately, receptor agonists or antagonists selective for various transmitter systems are not all available for use in humans. An animal model based on the human model of CCK₄-induced panic attacks may allow for preliminary investigations with new compounds before they come available for human use. The group of Ervin investigated the behavioural effects of CCK₄ in African green monkeys, which ranged from an increase in vigilance to immobility and freezing [77]. Immobilization and freezing reactions have been considered as equivalents of human fear or panic [11]. Therefore, the behavioural action to CCK₄ may provide a novel model of anxiety in primates for neurobiological studies.

Serotonergic Agonists

Direct tests of serotonin in man are complicated by a wide diversity of 5-HT receptor subtypes [76]. However, several attempts have been made to probe serotonin function in anxious patients. It would appear that increasing serotonin functions may result in increased anxiety although this is usually accompanied by other symptoms especially nausea. In man, *m*-chlorophenylpiperazine (mCPP), a direct agonist at 5HT₁- and 5HT₂-receptors, induces anxiety. The earliest study conducted by Charney and colleagues did not demonstrate differences in sensitivity between patients and controls in response to the 5HT agonist mCPP, probably due to a high dose of drug and resulting high levels of anxiety [25]. A separation in response to mCPP has been observed by Kahn and co-workers [63]. Panic patients showed increased anxiogenic and cortisol responsiveness as compared to controls, suggesting enhanced central 5HT receptor sensitivity. Hormonal responses to mCPP appear to support this hypothesis as mCPP causes greater release of cortisol in patients with panic disorder than in controls [64]. Administration of fenfluramine, a 5HT-releasing drug, provides further evidence to complement the hypothesis of hypersensitive serotonin receptors. Fenfluramine induces anxiety and panic attacks and elevated release of cortisol in patients with panic disorder but not in those with depression nor in healthy subjects [101]. By contrast, increasing serotonin function with the precursor L-tryptophan is rather sedating than anxiogenic [28]. It has been suggested by Nutt that augmentation of presynaptic serotonin function is anxiolytic, whereas postsynaptic potentiation is anxiogenic [76]. This hypothesis is contradictory to studies with inhibitors of 5-HT reuptake such as fluvoxamine in patients with panic disorder demonstrating biphasic responses; that is, an initial exacerbation of symptoms followed by amelioration [62]. Such data support the view that increasing serotonin function is anxiogenic, and suggest that the adaptation which presumably occurs on repeated administration of SSRIs accounts for their anxiolytic efficacy.

Yohimbine

In view of possible involvement of noradrenergic neurons of the locus coeruleus in anxiety-like behaviour of ani-

mals norepinephrine has been the most extensively studied monoamine in anxiety [67]. Various attempts of experimental manipulation of brain norepinephrine function have been made with adrenergic agonists and antagonists [76]. Yohimbine, an α ₂-adrenergic antagonist, reduces presynaptic inhibition and increases norepinephrine release from central as well as peripheral noradrenergic neurons. It has been noted to induce panic attacks and produce greater increases in anxiety in patients with panic disorder as compared to those with generalized anxiety disorders or controls [2, 26, 113]. The anxiogenic action of yohimbine can be attenuated by benzodiazepines and selective serotonin inhibitors [24, 39].

Caffeine

Oral administration of caffeine (10 mg/kg) to healthy humans can provoke increases in anxiety [23]. Uhde and colleagues compared various doses of caffeine up to 720 mg (equivalent to 8 cups of coffee) and found dose-effect relationship between the amount of caffeine administered and anxiety measured by subjective rating [104]. Patients with panic disorder appear to be more sensitive to the anxiogenic effects of caffeine than normals [12]. Indeed, many panic patients put themselves on a caffeine-free diet because they find the subjective effects of caffeine unpleasant. Bruce and Lader have reported that caffeine abstinence can ameliorate symptoms of anxiety disorders [22]. The central pharmacological profile of caffeine has not yet been defined. One study in mice suggested that caffeine might be a benzodiazepine receptor ligand [108] but this assumption was questioned in other studies [27]. It is agreed that caffeine acts as an antagonist of adenosine receptors [95, 100]. Investigation of polymorphisms of adenosine receptor genes in healthy humans suggested a role of one of the receptor genes in caffeine-induced anxiety [3]. Adenosine receptors were found to mediate the anxiolytic effect of ethanol in mice [87]. An association between central norepinephrine neurotransmission and caffeine-induced anxiety has been postulated by Berkowitz and colleagues [9]. It was noted by them that caffeine could increase the release of norepinephrine in the brain. Should this be the case in humans then this would be in line with the theory of norepinephrine over activity in anxiety.

USE OF PSYCHOLOGICAL MEANS

Fear Potentiated Startle

The startle reflex is a response to a sudden intense stimulus sensitive to aversive states. Rodents show robust startle potentiation, i.e. the jump response of rats to a loud noise can be potentiated in the presence of an aversive conditioned cue, that is a previously neutral stimulus that has been paired with an electric foot shock or another primarily aversive stimulus [20]. Grillon and colleagues adapted this model for use in humans [50]. They recorded the eye blink reflex elicited by a loud noise and potentiated by a threat cue (red light) signaling an impending aversive event (electric shock). The magnitude of the startle response was used as to index the degree of fear or alarm experienced during the periods of threat and safety. More sustained forms of startle potentiation can be found when the experimental room is in complete darkness, or following context conditioning [52]. It has been suggested that this sort of test provokes conditioned, antici-

patory anxiety, suitable to model generalized anxiety disorders [46]. However, studies on the effects of benzodiazepine anxiolytics on fear potentiated startle reactions have yielded contradictory results. Baas and co-workers reported 4 studies which did not detect any influence of lorazepam and diazepam on fear potentiated startle to a threat cue [5]. By contrast, others have shown that alprazolam, diazepam and lorazepam can reduce fear-potentiated startle in threat of shock experiments [10, 48, 90]. A negative result has been obtained from a study conducted with ethanol [29]. An enhanced fear potentiated startle reaction is noted in subjects with high levels of trait anxiety, and in offsprings of patients with anxiety disorders [49, 53]. Fear potentiated startle may thus detect vulnerability to anxiety disorders but does not seem very specific.

Skin Cutaneous Responses to Stressful Stimuli

Anxiety is accompanied by an increase in activity of sweat glands which are innervated by cholinergic fibres of the sympathetic nervous system. Autonomic innervation of sweat glands is reflected in changes in skin conductance at the surface, termed electrodermal activity (EDA). EDA incorporates both slow shifts in basal skin conductance (SCL) and more rapid transient events, that is skin conductance responses (SCRs) which are sensitive to the effects of centrally active drugs on the autonomic nervous system [96, 98]. Recording of SCRs to stimuli can be used to measure conditioned anxiety in healthy humans. Vila and Beech developed a procedure of aversive conditioning measuring SCR elicited by a blue light before and after its pairing with loud white noise [110]. It was demonstrated by them that the association increased the magnitude of SCR, an indication of classical conditioning. In subsequent studies tones were used instead of light [55, 111]. Habituation and extinction of SCRs can be separated from each other by presenting tones and noises at pseudo-random intervals. Diazepam facilitates both habituation and extinction whereas ritanserin, a 5-HT₂-receptor antagonist, selectively increases extinction. Aversive conditioning to tones is viewed as a paradigm of conditioned anxiety. Drugs which decrease the action of 5HT have been suggested to inhibit aversive conditioning. This hypothesis is in line with the effect of ritanserin on extinction of SCRs mentioned. It is further supported by studies demonstrating that the anxiogenic serotonergic agonist mCPP and fenfluramine facilitate conditioning [45]. However, the anxiolytic effects of the 5HT₁-receptor agonist buspirone and the selective 5-HT reuptake inhibitor fluvoxamine are discordant findings [59]. Anxious neurotic patients have higher skin conductance levels and response amplitudes to tones than controls but do not differ in habituation nor in conditioning when compared to normals [4]. Patients with panic disorder show more spontaneous fluctuations of skin conductance than controls but conditioning of skin conductance responses is similar in both groups [33]. In view, of these findings, it has been suggested that panic patients seem to process unconditioned fear abnormally rather than conditioned anxiety.

Stroop Colour-Word Test

The Stroop Colour-Word test was originally designed to test basic cognitive functions in humans. In 1935 it was observed by Stroop that naming the colour of words appearing

in another colour takes more time than naming the colour of words appearing in the same colour. This phenomenon became known as Stroop's interference. The procedure produces a cognitive conflict that may induce anxiety [103]. Word cards as well as computerized and video versions of the test are available. For the test, the subject is asked to name aloud or write colour words appearing in colours congruent and incongruent with their meanings as fast as possible. Errors are pointed out to them; the frequency of errors and the time needed to perform the test are recorded [97]. It is assumed that psychological stress induces errors and slows the test performance. It has been demonstrated that state anxiety induced by the Stroop procedure is attenuated in subjects with high levels of trait anxiety (>50) by diazepam and nebilone, a synthetic cannabinoid receptor ligand with anxiolytic properties, whereas those with low levels of trait anxiety do not respond to anxiolytic treatment [75]. Lorazepam was shown to suppress increases in heart rate but did not influence state anxiety nor skin cutaneous responses induced by the Stroop procedure [102]. Further studies are needed to evaluate the validity of the Stroop test as a model of anxiety in humans.

Simulated Public Speaking

Fear of speaking in public is the most frequent fear which can be found patients with social phobia [21], and is also highly prevalent among students [38]. In the simulated public speaking test the subject is asked to prepare a speech and subsequently speak in front of a video camera when being video taped. Measures of anticipatory anxiety are taken before speech onset. The speech is interrupted for a short time in order to assess stress-induced anxiety. Each subject participates only once. The majority of studies with benzodiazepines demonstrated an attenuating effect of the anxiolytics on anticipatory anxiety but could not show any influence on the increase in anxiety measured during simulated public speaking [47, 56, 57, 73, 115]. By contrast, agents which influence the serotonergic neurotransmitter system do not affect anticipatory anxiety. They either increase (clomipramine, nefazodone) or decrease (fenfluramine) stress-induced anxiety [57, 60, 99]. Patients with panic disorder show lower levels of anticipatory anxiety and lower increases of anxiety induced by simulated public speaking when compared to normals. [78]. The differences between both groups are reduced when panic patients receive chronic treatment with a serotonin reuptake inhibitor. It has therefore been hypothesized that panic patients who are symptomatic process the type of anxiety (unconditioned fear) generated by the simulated public speaking test in an abnormal way [33].

DISCUSSION

The objectives and process of drug development have been changing over the last 10 years. Due to increasing costs and market competitiveness companies can no longer afford to continue to late phase III with drugs which are unlikely to be therapeutically effective, or to market new products which lack superiority over existing treatments. For anxiety disorders clinical trials are difficult due to a huge placebo effect and sometimes apparently adequately powered clinical studies may fail to demonstrate the known effect of an anxiolytic drug. Thus, there is an increasing pressure to determine the

likely probability of therapeutic and commercial success of a novel anxiolytic compound as early as possible in drug development.

Exploratory drug development consists of all work required to demonstrate the likely chance of therapeutic success. Sometimes this is referred to as 'proof of principle' [91]. The objectives of exploratory development are to demonstrate dose- and concentration response relationships for desired and undesired effects. This approach is particularly important to small pharmaceutical and biotechnological companies. Such companies frequently do not have the resources to take a new drug to the market, and need to attract a development partner. The success in attracting a partner will depend on whether the 'proof of principle' point has been achieved. A model of anxiety is an experimental system or paradigm to simulate some aspects of anxiety disorders in which the effects of anxiolytic drugs are examined. One major difficulty confronted in experimental models of anxiety is the lack of well validated parameters reflecting anxiety. By analogy to depression and pain, no specific operational measure of anxiety exists. Surrogate markers such as an increase in skin conductance level and motor responses following stressful stimulation can be instructive in the evaluation of anxiety in humans. However, the autonomic and somatic variables do not reflect the role of cerebral mechanisms in the modulation of anxious states. Therefore, subjective rating scales of state and trait anxiety are employed as primary and or secondary end-points.

The use of provocative chemicals to induce anxiety states in humans has contributed to some extent to the understanding of panic attacks. However, the provocative agents available are unspecific which limits their use to explore neurotransmitters involved in anxiety disorders. Thus, anxiety induced by chemical means in healthy subjects or in patients may provide rather a screening instrument to test anxiolytic compounds than a specific tool for early stages of clinical drug development. From the reviewed evidence on psychological means to induce anxiety one may interfere that conditioned autonomic responses and fear potentiated startle on the one hand and simulated public speaking on the other hand generate different anxiety states. The conditioned anxiety induced by the former models is sensitive to benzodiazepines to a certain extent and appears to be related to generalized anxiety disorder. By contrast, the anxiety generated by simulated public speaking is likely unconditioned, resistant to these drugs and appears to be related to panic disorder. The status of the Colour Word Stroop test is still unclear.

The failure of some studies to find an effect of benzodiazepines, the major class of anxiolytics, on anxiety models such as fear potentiated startle may question their validity to test efficacy of anxiolytic in humans [51]. One should differentiate between an experimental model to induce an aversive emotional state and the method to measure aversive state. The fact that potentiated startle to an explicit threat signal is not reduced by benzodiazepines does not necessarily mean that the model is not sensitive to benzodiazepines. It means that fear to an imminent shock is not alleviated by benzodiazepines. As pointed out above, imperfect validation and lack of specificity may limit the usefulness of various models

of anxiety in humans. But, decision making in drug development is rarely based on a single piece of data. Models of human experimental anxiety, even though their predictive power is often incompletely understood can be used to support the decision to commit to a major clinical trial program and to assist with dose selection. Development of valid models and surrogates may come from advances in the understanding of basic mechanisms of anxiety disorders but such models need to be prospectively evaluated by people experienced in designing and testing methods of drug action in man.

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