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Review

GABA-A receptors and the response to CO_2 inhalation — A translational trans-species model of anxiety?

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ABSTRACT

The mechanisms by which the inhalation of carbon dioxide (CO_2) produces anxiety and panic are not fully understood, although more recently there is evidence to suggest the involvement of a neural 'fear circuit'. We have suggested that this neural fear circuit is partly mediated by the brain noradrenaline network [Bailey, J.E., Argyropoulos, S.V., Lightman, S.L. and Nutt, D.J., (2003) Does the brain noradrenaline network mediate the effects of the CO_2 challenge? J Psychopharmacol 17(3): 252–259.]. However, we now review evidence that GABA-A may also play an important role in the modulation of CO_2-induced anxiety.

The review of this evidence starts with a key publication showing that 1 min of 35% CO_2/65% air produced anxiogenic effects in a rat model of anxiety, to a similar extent to the anxiogenic betacarboline derivative FG7142, a benzodiazepine receptor inverse agonist. The effects of both anxiogenic stimuli were abolished with pre-treatment with alprazolam (0.5 mg/kg), but only those of FG7142, not CO_2, was blocked by a benzodiazepine antagonist [Cuccheddu, T., Floris, S., Serra, M., Porceddu, L., Sanna, E., Biggio, G., (1995) Proconflict effect of carbon dioxide inhalation in rats. Life Sci 56: PL 321–324.]. Although the evidence from this study did not conclusively prove that CO_2 had an action to reduce GABA function, it was an experiment designed to be translational to compare what was known about CO_2-induced anxiety in patients, and to also to explore if GABA mechanisms are involved.

Additional evidence from the literature is found in the association between GABA and chemoreceptors, both in laboratory and human studies and GABA and anxiety disorders. Evidence of this association is found across species from stress-induced change in GABA levels in plants and insects to humans, where there is now much evidence of abnormalities in GABA/benzodiazepine receptors in anxiety and other psychiatric disorders. This paper reviews some of the evidence and attempts to relate and compare these findings across species from the human to the *Drosophila*.

Contents

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1. Introduction

The inhalation of hypercapnic gas has been used in psychiatric research for decades. Since the early 1980s however, the inhalation of CO_2 as a challenge in humans has focussed mainly on the provocation of panic attacks in patients with panic disorder and this has been variously described as a diagnostic tool, or as a means to explore the mechanisms involved in the production of panic attacks. Much of this research originated from Klein's theory suggesting that panic disorder patients panic because they have increased sensitivity of the central chemoreceptors to carbon dioxide and thus are more likely to hyperventilate and trigger the 'suffocation false alarm', which in turn leads to panic (Klein, 1993).

This theory has been tested with carbon dioxide challenges to examine and compare ventilatory response and respiratory variables in patient populations and volunteers by a number of groups (e.g. Gorman et al., 1988; Lousberg et al., 1988; Pain et al., 1988; Papp et al., 1995; Papp et al., 1997). These studies have yielded different and inconclusive results. However, more recently, Gorman et al. (2001) felt it important to conduct a further study with an independent cohort of patients to assess whether increased ventilatory response is patient-specific, i.e. are only patients with panic disorder sensitive or is any person who panics, regardless of diagnosis, more sensitive? Patients with a diagnosis of panic disorder, major depression, pre-menstrual dysphoric disorder and normal volunteers underwent the 5% and 7% CO_2 challenge. Continuous respiratory measures of tidal volume, respiratory rate, minute volume and end tidal CO_2 were conducted. The authors concluded that having a panic attack in response to CO_2 was more important than having a diagnosis of panic disorder in distinguishing response and that rather than, or in addition to, abnormal chemoreceptors, central brain circuits are implicated. They propose a theory that 'panic' to CO_2 involves a more generalised fear response implicating the amygdala and other parts of a neural 'fear' circuit.

We have suggested that this neural fear circuit is partly mediated by the brain noradrenaline network (Bailey et al., 2003). However, we now review evidence that GABA-A may also play an important role in the modulation of CO_2-induced anxiety. Key to this is a brief communication published in 1995 supporting a role for GABA-mediated transmission in the anxiogenic effect of CO_2 inhalation in rats (Cuccheddu et al., 1995). They showed that being exposed to 1 min of 35% CO_2/65% air significantly decreased the number of licking periods in the proconflict test indicating increased anxiety, to a similar extent to the anxiogenic betacarboline derivative FG7142, a benzodiazepine receptor inverse agonist. The effects of both anxiogenic stimuli (gas and FG7142) were abolished with pre-treatment with alprazolam (0.5 mg/kg), but only those of FG7142, not CO_2, was blocked by intraperitoneal flumazenil (5 mg/kg), a benzodiazepine antagonist. This study followed on from earlier observations that CO_2, foot shock and FG7142 decreased the function of the GABA-A receptor complex in rat brain (Concas et al., 1993). Although the evidence from these studies did not conclusively prove that CO_2 had an action to reduce GABA function, it did provide a translational model with which to compare what was known about CO_2-induced anxiety in patients, and also to explore if GABA mechanisms are involved.

Other forms of stressful stimuli in rat models have similarly demonstrated decreased GABA-A receptor binding. The effects of acute handling stress reduced the number of benzodiazepine receptors as demonstrated by lower [3H]flunitrazepam binding in the frontal cortex (Andrews et al., 1992), which was not seen with acute administration of diazepam, or habituation to handling. Also chronic restraint stress leads to reduced GABA-A receptor binding in prefrontal cortex, although it was unclear whether this was due to an increase in the Kd, a decrease in the Bmax, or both (Gruen et al., 1995). A more recent study has shown that social isolation produces increased anxiety in the elevated plus maze and that this was associated with decreased neuroactive steroids and GABA-A receptor function (Serra et al., 2000).

So is an increased concentration of CO_2 acting as a direct stressor to the central benzodiazepine/GABA receptor complex and if it is, what further evidence is available to support this hypothesis?

2. GABA and chemoreceptors — laboratory studies

In our previous paper (Bailey et al., 2003); we hypothesised that during hypercapnia, CO_2 acts directly at medullary chemoreceptors and peripherally at carotid body receptors to increase respiration and arousal. Activation of central noradrenergic neurones leads to an increase in blood pressure and these in turn act on the cortico-limbic circuit to produce the subjective sensation of increased anxiety or fear. The ventrolateral surface of the medulla functions as an area of central chemoreception and is involved in mediating the ventilatory response to hypercapnia (Loeschcke, 1982). In our studies in healthy participants and patients with generalised anxiety disorder respiratory rate as well as heart rate and blood pressure are significantly increased after exposure to CO_2.

Several eloquent physiological studies have examined medullary neuronal firing in the presence and absence of hypercapnia and have demonstrated a role for GABA-A receptors. Messier et al. (2002) showed that in newborn piglets a 5% CO_2 challenge increased medullary raphe neuronal activity and that this CO_2 response could be partially inhibited with the GABA-A receptor agonist muscimol. In addition, 5% CO_2 reversed muscimol-induced sleep. At normal oxygen levels 10% CO_2 resulted in a significant increase in c-Fos expression in GABA-containing neurones in the medulla, especially the ventral aspect, in week old piglets (Zhang et al., 2003). The authors suggest that this data, though not definitive, indicates that these medullary cells are part of the chemosensory network that is involved in responses to hypercapnia. These GABAergic neurones along the ventrolateral aspect of the medulla include a subset of neurones known as the Botzinger complex, which is essential to the generation of respiratory rhythm in mammals (Smith et al., 1991). It is proposed that GABAergic neurones in the neural network regulating inspiratory drive and respiratory timing respond to hypercapnia to stimulate respiration and this is presented in a schematic model and discussed in detail (Zhang et al., 2003).

A further study tested the hypothesis that during hypercapnia, partial removal of a tonic GABA-mediated inhibition plays a role in the increase in activity of the ventrolateral medulla inspiratory neurones (Gourine and Spyer, 2001). They investigated the effects of the GABA-A receptor antagonist bicuculline on the electrical activity of the ventrolateral medullary inspiratory neurones of rats during normo- and hypercapnia. The results suggested that both hypercapnia and bicuculline changed the firing frequency and discharge pattern of the inspiratory neurones to a similar degree, but bicuculline was not additive to the effect of hypercapnia alone and they concluded that modifications of GABA-inhibitory inputs are essential features of the chemosensory control of respiratory activity.

We previously mentioned that increased levels of CO_2 act directly at the peripheral chemoreceptors of the carotid body. It is known that anaesthetics acting at the GABA-A receptor, such as diazepam and midazolam, cause respiratory depression. In a study to examine whether midazolam at clinically relevant doses depresses carotid body chemoreceptor activity, Kim et al. (2006) demonstrate a dose-related depression of carotid body chemoreceptor activity. Since midazolam has high affinity for the GABA-A receptor, this study shows that peripheral as well as central chemoreceptors are involved in the response to elevated levels of CO_2.

Of course GABA is quantitatively the most important inhibitory transmitter in the CNS and controls the state of excitability in all brain areas. Neuronal activity is regulated by a balance of excitatory (mainly glutamatergic) and inhibitory GABAergic activity. In evolutionary terms it is thought that GABA receptors are necessarily available to reduce anxiety. Indeed endogenous benzodiazepine agonists have been identified and GABA is found in virtually all plant tissues (see Nutt and Maliza, 2001). Several studies show that GABA levels in plants increase in response to a variety of stress conditions (see Crawford et al., 1994). Interestingly one of these stimuli is hypoxia which enhanced GABA synthesis in ammonium-pre-treated maize root tips (Roberts et al., 1992).

To continue the theme of cross-species translational research, it is reported that in *Drosophila* CO_2 elicits avoidance behaviour and that this may be mediated via highly specific olfactory circuitry (Suh et al., 2004). A separate study has revealed the involvement of GABAergic involvement in olfactory processing (Wilson and Laurent, 2005) and they revealed specific roles of GABA-A and GABA-B in odour responses. A more recent study has identified specific chemosensory neurones in *Drosophila* (Kwon et al., 2007), but as yet, there is no direct relationship between these chemosensitive neurones and GABA. However, since molecular studies in *Drosophila* have identified polymorphisms of the human homologue associated with mood and panic disorders (Nakamura et al., 1999), the CO_2 model across species offers exciting possibilities.

3. GABA and 5-HT

We have previously discussed the importance of noradrenergic pathways in the response to CO_2 (Bailey et al., 2003) and although the evidence for GABA-A to be contributory to the mechanisms associated with CO_2-induced anxiety is compelling, we must also consider the role of 5-HT. This will not be discussed in this review in depth, however, it must be noted that serotonergic and gabaergic neurones coexist in the raphe nuclei (Gao et al., 1993; Judge et al., 2006) and that these serotonergic neurones are also involved in anxiety, stress and the sleep/wake cycle. Richerson et al. (2001) describe chemosensitivity of serotonergic neurones in the rostral ventral medulla, as described previously an area important in respiration. Another brain area involved in aversion is the dorsal half of the midbrain periaqueductal grey (dPAG) and studies by Griffiths and Lovick (2002) have shown that the majority of 5-HT_{2A} receptor labelled cells also showed immunoreactivity for GABA.

Johnson et al. (2005) investigated the effect of exposure to elevated atmospheric concentrations of CO_2 on the serotonergic systems in rats. They revealed an increase in c-Fos activity within serotonergic cell groups with the ventrolateral periaqueductal grey and ventrolateral part of the dorsal raphe nucleus, which are regions associated with fight or flight behaviour.

These studies demonstrate that 5-HT and GABA processes are involved in the anxiogenic response to CO_2 which is intriguing in that the two main treatment groups that are used clinically to treat patients with anxiety disorders are the benzodiazepines and the serotonin reuptake inhibitors. The next section will review the human studies of CO_2-induced anxiety and attenuation of symptoms with drugs.

4. GABA and anxiety disorders

There is now much evidence of abnormalities in GABA/benzodiazepine receptors in anxiety and other psychiatric disorders. Key evidence is described in a study by Nutt et al. (1990), showing that when patients with panic disorder were given intravenous flumazenil (2 mg), a benzodiazepine antagonist, it produced a panic attack in most of the patients. This was not the case in the matched controls, who experienced no panic or anxiety symptoms. At this time, the hypothesis put forward was that in patients there is a shift in the “set-point” of the benzodiazepine spectrum so that the effects of full benzodiazepine agonists are reduced in patients compared with healthy normals and that the antagonist flumazenil becomes a weak inverse agonist thus producing anxiety (Nutt and Maliza, 2001).

However, further and more recent evidence of these GABA/benzodiazepine receptor abnormalities come from imaging studies. There is a localised reduction in benzodiazepine binding in generalised anxiety disorder (Tiihonen et al., 1997) and a more global reduction of benzodiazepine receptor binding in untreated panic disorder patients (Malizia et al., 1998) and in PTSD (Bremner et al., 2000). A more recent study using proton magnetic resonance spectroscopy has reported decreased GABA levels in anterior cingulate and basal ganglia in medicated patients with panic disorder compared with healthy controls (Ham et al., 2007). Abnormalities in GABA/benzodiazepine binding have also been reported in post-mortem studies of mood disorders (bipolar disorder and major depression) (Bielau et al., 2007), fMRI studies in alcoholism (Schlösser et al., 2007), proton magnetic resonance spectroscopy in major depression (Hasler et al., 2007), and post-mortem studies in schizophrenia (Newell et al., 2007).

Neurosteroids are released in response to stress and anxiety (Le Melledo and Baker, 2004) and have central effects through interactions with neurotransmitter receptors (Rupprecht et al., 2001). They act as endogenous anxiolytics through interactions with GABA-A receptors to restore equilibrium (Strous et al., 2006). The effects of neurosteroids have been likened to that of barbiturates and benzodiazepines (Reddy, 2003) and have GABA receptor affinity more potent than barbiturates and benzodiazepines (Rupprecht et al., 2001).

There is emerging evidence of the interaction between neuroactive steroids and GABA in the development of neuropsychiatric disorders in women, particularly across the menstrual cycle, during and post-pregnancy and at peri- and post-menopausal time (Amin et al., 2006; Paoletti et al., 2006; N-Wihlbäck et al., 2006). As yet, there have been no studies of CO_2-induced changes in neurosteroid levels, however, in patients with panic disorder, sodium lactate and cholecystokinin-induced panic significantly reduced allopregnenolone and pregnenolone levels. The hormones were at their lowest levels at the peak panic symptomatology for the group having sodium lactate (Strohle et al., 2003). In healthy controls no effect was seen on hormone levels or measures of anxiety (Strohle et al., 2003; Zwanzger et al., 2004).

5. CO_2 and anxiety disorders

Much of the published literature on CO_2 inhalation and anxiety stems from the serendipitous discoveries from Gorman et al. (1984) and van den Hout and Griez (1984). Gorman et al. (1984) used 5% CO_2 as a control gas for an experiment in which panic disorder patients hyperventilated. At that time it was believed that hyperventilation caused panic attacks, but Gorman et al. discovered that more patients panicked in the CO_2 group than the hyperventilation group. The discovery that inhalation of 5% CO_2 produces panic in panic disorder patients, but not in subjects without anxiety, has been replicated and validated and today is a well recognised experimental model.

van den Hout and Griez (1984) were also experimenting with CO_2 inhalation. They were using a 35% CO_2 inhalation as a behavioural exposure paradigm to teach patients to deal with their anxiety and panic attacks. However, gas inhalation increased autonomic symptoms and feelings of anxiety and panic in panic disorder patients. This initial experiment led to further exploratory studies in patients and volunteers and today the 35% single inhalation technique of anxiety provocation is reliable and well documented (Verburg et al., 2001).

Both methods of CO_2 inhalation appear to be reliable in producing panic symptoms in patients with panic disorder. However, the extent to which anxiety and panic symptoms are produced in patients with other anxiety disorders and healthy subjects is less well documented. Many different rating scales are used to assess ‘response’ to the inhalation and most are ‘panic-specific’. In addition there is no consistent interpretation of what determines a ‘panic attack’, so it is therefore difficult to interpret the published findings from different groups.

To demonstrate this difficulty in interpretation we re-interpreted published data from 2 reviews. First Verburg et al. (1998) who examined whether the 35% CO_2 challenge could be used as a diagnostic test for panic disorder, using pooled data from the Maastricht and Milan centres giving a database of 549 challenges. This paper pulls together information on the response to 35% CO_2 of patients with anxiety disorders and normal controls. The scores obtained from the Panic Symptom List (PSL) are reported in Verburg et al. (1998), but here in Table 1 are expressed as a percentage of maximum possible score and in order of apparent patient group sensitivity.

Table 1 shows that although patients with anxiety disorders score higher than normal controls on the PSL in response to 35% CO_2, there appears to be little difference between the groups. This suggests that the PSL is poor at discriminating between panic disorder patients, other anxiety disorders and normal controls, or perhaps the disorders are not specific and general CO_2-induced symptoms are being detected. Indeed, the authors conclude that the best discriminator is probably the use of a simple visual analogue rating scale.

The second comparison was made with data from Rapee et al. (1992) who used a 15-point version of the PSL; the Diagnostic Symptoms Questionnaire (DSQ). Items are rated on a 9 point scale, 0 = none and 8 = very strongly felt, thus presumably giving a maximum total score of 120 representing a severe full-blown panic attack. DSQ data were collected in response to inhalation of 5.5% CO_2 in different patient groups and this published data has again been expressed as a percentage of maximal score and outlined in Table 2, again presented in order of apparent sensitivity.

From these two sets of re-interpreted data, it appears that the DSM panic symptoms produced by 5.5% CO_2 challenge are less severe than produced by the 35% CO_2 challenge. Alternatively, the use of a 9 point scale to rate panic symptoms is a lot less sensitive than the 5 point scale.

Other studies in patient groups have examined the response to CO_2 in greater detail. The early studies of Woods et al. (1986, 1989) considered ventilatory and anxiogenic responses to 5 and 7.5% CO_2 in patients with panic anxiety and healthy subjects and reported attenuation in response after alprazolam and discuss possible mechanisms involving the stimulation of benzodiazepine receptors located on noradrenergic neurones. It has been noted that the sensitivity to 35% CO_2 differs across the menstrual cycle in panic patients (Perna et al., 1995), where CO_2-induced anxiety was stronger in the early follicular phase than the midluteal phase. This was not the case in healthy controls, although numbers in both groups were small.

More recently, studies have revealed that there is an increased sensitivity to CO_2 inhalation in healthy first-degree relatives of patients with panic disorder (van Beek and Griez, 2000). Indeed a recent paper has confirmed that genetic factors can explain most of the individual differences in reactivity to hypercapnia, as assessed by the 35% inhalation technique (Battaglia et al., 2007). Although the findings from this study do not confirm a definite endophenotype for definition of panic disorder, it does suggest an association between response to CO_2 and history (familial or personal) of panic, agoraphobia or social phobia.

The published literature also shows a number of studies that reveal greater sensitivity to the inhalation of CO_2 in patients with a psychiatric diagnosis. Unfortunately, not all of these studies have a matched control group and again assessment of response to CO_2 is not consistent between studies. Gorman et al. (2001) performed the 5% or 7% CO_2 challenge on patients with panic disorder, patients with pre-menstrual dysphoric disorder, patients with major depression and normal controls. They found that more patients than volunteers experienced a panic attack in response to CO_2, regardless of diagnosis and that once panic was triggered; the physiological features were similar across groups.

Abstinent alcohol dependent individuals are hypersensitive to the effects of CO_2 in the Read's Rebreathing technique (Read, 1967; Rassovsky et al., 2004), as are patients with bipolar disorder with 5% CO_2 inhaled for 15 min (MacKinnon et al., 2007). However, there is no reported difference in response to 35% CO_2 in patients with panic disorder, with or without a history of respiratory pathology (Van Beek et al., 2003) and patients with eating disorder (Perna et al., 2004) or post-traumatic stress disorder (Talesnik et al., 2007) do not appear to show a hypersensitivity to inhaled CO_2. There are conflicting reports of whether patients with social anxiety disorder (social phobia) are equally sensitive to CO_2 as patients with panic disorder. However, it seems that sensitivity to CO_2 is equivalent in most studies using 35% and 5% inhalations (Gorman et al., 1990; Holt and Andrews, 1989; Caldirola et al., 1997) with only one showing that panic disorder patients are more sensitive to the effects of 35% CO_2 than patients with social phobia (Papp et al., 1993).

To support our recent observations that the inhalation of 7.5% CO_2 in healthy participants reproduces some of the symptoms seen in patients with GAD, we studied 10 unmedicated patients with a diagnosis of GAD. The inhalation procedure consisted of 2 study days, a week apart when a 20 min inhalation of air and a 20 min inhalation of 7.5% CO_2 were administered on each occasion. The inhalation of CO_2 significantly increased subjective anxiety and other symptoms, compared with air on both study days and these ratings were of a similar magnitude to those observed in healthy participants without a diagnosis of GAD (Seddon et al., 2007).

Interestingly there is one report that patients with specific phobias also show an increased sensitivity to the effects of CO_2 (Antony et al., 1997), something that we have also observed in our studies of CO_2 inhalation in normal volunteers when a panic response has occurred unexpectedly. On the limited occasions this has occurred, further questioning has revealed a previous anxious response to blood, needles or heights which was not detected at screening.

6. CO_2 in healthy volunteers

We have now performed over 200 study sessions using the inhalation of 7.5% CO_2 in healthy volunteers and much of this work is published (Nutt and Bailey, 2002; Argyropoulos et al., 2002; Bailey et al., 2003, 2005, 2007a,b, in press). Briefly, two 20 min inhalations are

Table 1
Response to 35% CO_2 measured by the PSL, in anxiety disorders and normal controls

Group	Number	PSL TSS Mean ± sd	% of max. score
Specific phobia	30	11 (7)	22
Panic disorder	318	11 (8)	21
Social phobia	31	11 (8)	21
GAD	17	10 (7)	20
OCD	30	8 (7)	15
Normal controls[a]	123	6 (6)	11

PSL = panic symptom list, TSS = total symptoms score.
[a] 17% of normal controls had a history of spontaneous panic attacks, thus this sample is representative of the normal population.

Table 2
Response to 5.5% CO_2 as measured by the DSQ (diagnostic symptoms questionnaire), in anxiety disorders and normal controls

Group	Number	DSQ score Mean ± sd	% of max. score
Panic disorder	35	7 (4)	6
GAD	33	5 (4)	5
Social phobia	38	5 (3)	4
OCD	25	5 (4)	4
Simple phobia	27	5 (3)	4
Normal controls	25	3 (3)	2

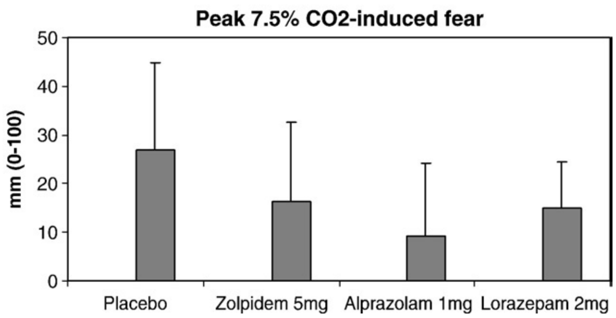

Fig. 1. Effects of single dose anxiolytics and placebo on healthy volunteers in the 7.5% CO_2 challenge. VAS Fear was assessed as peak effects of gas. $N=12$ for all groups. Alprazolam and lorazepam, but not zolpidem are statistically significantly different from placebo.

delivered via an oro-nasal face mask and gas is delivered via piping from a reservoir attached to the gas cylinder which is kept out of sight. We always use air as a control gas and the normoxic hypercapnic gas is CO_2 7.5%/21% O_2 and nitrogen. Gases are delivered in a single-blind order, but the participant is not aware of this and expectation effects are equal in all studies, even though the CO_2 is delivered after air.

A 20 min inhalation of 7.5% CO_2 produces reproducible increases in fear, anxiety, tension, worry and reduces feelings of being happy and relaxed. It also increases heart rate, blood pressure and respiratory rate. This does not lead to a panic attack, but a heightened general anxiety, which contrasts with the more acute anxiety produced with a vital capacity inhalation of 35% CO_2. This has led us to the hypothesis that 7.5% CO_2 is more of a model of generalised anxiety disorder than panic disorder. This is discussed more fully in Bailey et al. as above. With these repeatable and robust findings it is appropriate to use the inhalation of CO_2 as models of human anxiety. However to validate this further, it is important to know whether medication used in the clinic can attenuate the CO_2-induced responses.

7. Effects of pharmacological manipulation

7.1. Benzodiazepines

Many studies in patients and fewer in normal volunteers have shown that drug treatments can attenuate the response to inhalation of CO_2. Arguably one of the best treatments for anxiety acutely is still the benzodiazepines. Several patient studies have demonstrated an attenuated anxiety response to 35% CO_2 after a single dose of the benzodiazepines, alprazolam, (Pols et al., 1996; Sanderson et al., 1994) and clonazepam (Valença et al., 2000). Pols et al. (1991) have also demonstrated that 5 weeks' treatment with clonazepam leads to a reduction in the 35% CO_2-induced response consistent with its anti-panic actions. Our recent studies for the validation of the 7.5% CO_2 model of GAD have shown efficacy of lorazepam 1 mg and 2 mg (Bailey et al., 2007a; Nutt and Bailey, 2002), alprazolam 1 mg and also zolpidem 5 mg, which is a GABA-A agonist selective for the alpha-1 subtype receptor (Bailey et al., in press). This reduction in CO_2-induced fear is shown in Fig. 1.

7.2. Other anxiolytics

The tricyclic antidepressants imipramine and clomipramine have produced an attenuated anxiety response to 35% CO_2, when given to panic disorder patients for 7 days (Bertani et al., 1997; Perna et al., 1997) and the serotonin reuptake inhibitors, which are effective anti-panic medications, have been studied. Paroxetine, sertraline (Bertani et al., 1997), fluvoxamine (Perna et al., 1997) and citalopram (Bertani et al., 2001) have all produced an attenuated response to 35% CO_2 in panic disorder patients. Our validation studies using 7.5% CO_2 have shown that in healthy volunteers, paroxetine 20 mg taken for 21 days reduces some of the symptoms produced (Bailey et al., 2007a), but no other studies have examined the effects of SSRIs and the CO_2 challenge in healthy participants. Moreover, given the evidence already presented in this review, we would like to propose that one of the primary mechanisms by which CO_2 produces anxiety is by reducing available GABA, both centrally and peripherally and that this mechanism occurs in tandem with the noradrenergic and serotonergic activation.

8. CO_2, anxiety and GABA

We have reviewed the literature that shows that in laboratory models hypercapnia produces changes in the GABA-A receptor complex. There is convincing evidence for this across species which is probably a fundamental evolutionary survival adaptation. We have presented evidence that humans are sensitive to the effects of CO_2 when they have an anxiety disorder, or have fluctuations in their neurosteroid levels. It is generally accepted that the GABA-A receptor complex is involved in the specific anxiety disorders that are sensitive to CO_2. In addition, anticonvulsant drugs are used in the treatment of bipolar disorder and the mechanism of action for many of the drugs in this class is via the GABA-A receptor complex. A recent review outlines the role of anticonvulsant drugs in anxiety disorders and discusses mechanism and evidence of treatment (Mula et al., 2007) and a recent paper has examined the anticonvulsant efficacy of GABA-A receptor subtypes of benzodiazepine site ligands in a mouse model (Fradley et al., 2007).

To our knowledge, no studies have yet examined the effects of anticonvulsant drugs on the CO_2 challenge in humans. However, another drug that is used by patients with anxiety disorders to self-medicate – alcohol – has been shown in one study to reduce the response to 35% CO_2 in patients with panic disorder and healthy volunteers (Cosci et al., 2005). Other papers in this Special Edition of PBB discuss the role of GABA-A and alcohol (Enoch, 2008-this issue; Lobo and Harris, 2008-this issue) and see Biggio et al., 2007 for a comprehensive review.

9. Future studies to test hypothesis

Recent pharmacological treatments have not been successful in the same way that the benzodiazepines were, and in order to find the 'ideal anxiolytic' and indeed the development of other drugs for CNS disorders, the pharmaceutical industry annually invests billions in research and development. However, GABA-A receptor subtype-selective drugs are now in early phase clinical studies and are showing selective efficacy in pharmacodynamic studies. For example the GABA-A alpha2,3 subtype-selective agonist TPA023 has been compared against placebo and lorazepam in healthy volunteers. Although TPA023 dose-dependently slowed saccadic eye movement

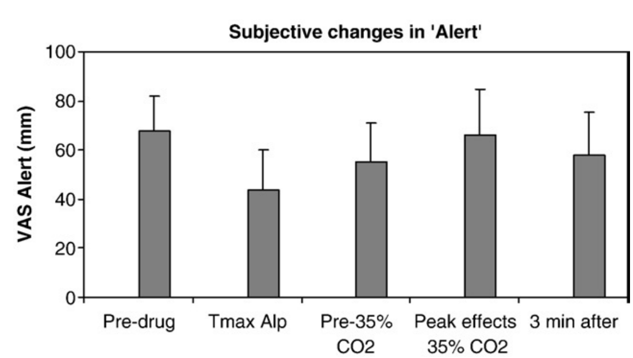

Fig. 2. Effects of single vital capacity inhalation of 35% CO_2 challenge on alprazolam 1 mg induced reduction in alertness. VAS Alert was assessed various time points before and after inhalation of gas. $N=12$. Data unpublished.

peak velocity, it did not appear to affect ratings of alertness, memory or body sway, unlike the full non-selective agonist lorazepam (de Haas et al, 2007 and see Lobo and Harris, and Enoch to add on this Special Edition). It would be interesting to examine the effects of this compound in the CO_2 human volunteer model of anxiety to test for potential anxiolytic activity as part of the drug development program.

Data from our studies suggest that 35% CO_2 reverses alprazolam-induced decreases in alertness (see Fig. 2), so is this percentage of inhaled CO_2 acting in the same way as a benzodiazepine antagonist or more likely inverse agonist? It would be interesting to conduct a comparative study in this way as a within-subject design in healthy volunteers as well as more comprehensive studies in animal models using a wider spectrum of subtype-specific GABA-A compounds against CO_2 or a benzodiazepine inverse agonist-induced anxiety, thus revisiting the original study of Cuccheddu et al. (1995).

10. Conclusion

We have presented evidence for a possible link between the GABA-A benzodiazepine receptor system and CO_2-induced anxiety across species from *Drosophila* to humans and have also highlighted the importance of using translational models. This evidence is not available for other methods of inducing anxiety in humans, where such translational comparisons could not be made, for example public speaking and mood induction. Further targeted translational studies would help to confirm these hypotheses and may also help in the search for safe and effective medication for clinical use.

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