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REVIEW

Metabotropic glutamate receptor modulation, translational methods, and biomarkers: relationships with anxiety

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Abstract
Rationale The increasing awareness of the need to align clinical and preclinical research to facilitate rapid development of new drug therapies is reflected in the recent introduction of the term "translational medicine". This review examines the implications of translational medicine for psychiatric disorders, focusing on metabotropic glutamate (mGlu) receptor biology in anxiety disorders and on anxiety-related biomarkers.
Objectives This review aims to (1) examine recent progress in translational medicine, emphasizing the role that translational research has played in understanding of the potential of mGlu receptor agonists and antagonists as anxiolytics, (2) identify lacunas where animal and human research have yet to be connected, and (3) suggest areas where translational research can be further developed.
Results Current data show that animal and human $mGlu_5$ binding can be directly compared in experiments using the PET ligand ^{11}C-ABP688. Testing of the $mGlu_{2/3}$ receptor agonist LY354740 in the fear-potentiated startle paradigm allows direct functional comparisons between animals and humans. LY354740 has been tested in panic models, but in different models in rats and humans, hindering efforts at translation. Other potentially translatable methods, such as stress-induced hyperthermia and HPA-axis measures, either have been underexploited or are associated with technical difficulties. New techniques such as quantitative trait loci (QTL) analysis may be useful for generating novel biomarkers of anxiety.
Conclusions Translational medicine approaches can be valuable to the development of anxiolytics, but the amount of cross-fertilization between clinical and pre-clinical departments will need to be expanded to realize the full potential of these approaches.

Keywords Anxiety · Translational models · Metabotropic glutamate receptor · Imaging · Fear-potentiated startle · Stress-induced hyperthermia · Biomarker · HPA axis · Allometric scaling

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The scientific and medical community has a long-standing interest in animal experimentation as a way to find cures for or alleviate symptoms of human diseases. The implicit understanding in the use of animals in medical research is that animal behavior and physiology in some way approaches that of humans. In recent years, this implicit understanding that data from animals can be translated to human research has been highlighted, and a new term introduced to research: translational medicine (Wehling 2006). The establishment of translational medicine acknowledges the critical importance of conducting research

in a manner that allows both moving from animal research to clinically relevant studies as quickly and smoothly as possible, and the possibility for preclinical research to learn from findings in humans.

Because we lack a good understanding of the pathophysiology of psychiatric disorders, classification schemes for psychiatric disorders have changed over time and are primarily based on symptom clusters, some of which are difficult or impossible to model in animals. Psychiatric disorders have traditionally been challenging to model in animals in a manner directly translatable to human research. However, with the growing emphasis on translational medicine, increasing efforts are currently being undertaken to align preclinical experimentation with psychiatric research. An example of this is seen in the field of anxiolytic development, in which paradigms that are well-characterized in animals have been translated for use in humans (e.g., fear-potentiated startle). Techniques previously used primarily in humans are also now available for use in animals, e.g., PET and MRI (Steckler et al. 2007). Examples of behavioral techniques used in translational paradigms are found in Table 1.

This shift in mindset bringing clinicians and preclinical scientists closer together is driven by the need to develop novel compounds with improved efficacy and side-effect profile for treating disease in all areas, including psychiatry. Current drug treatment for anxiety is focused on the prescription of benzodiazepines, with their unfavorable side-effect profile and potential for dependency, or antidepressants like selective serotonin reuptake inhibitors (SSRIs), which have delayed onset of action and undesirable side effects such as sexual dysfunction (Gale and Davidson 2007). Although both treatments are effective in some patients, between 20% and 50% of patients do not respond to drug treatment depending upon the specific anxiety disorder (Denys and de Geus 2005). Thus, there is still room for improvement in treatment of anxiety, both in terms of side effects and response rates. Current efforts are largely focused on uncovering and developing new drugs that bind to novel targets for treating anxiety, with an additional need to develop new translational approaches for these targets.

Table 1 Examples of "translatable" paradigms in anxiety research

Construct	Test
Psychosocial stress	Public speaking (human); psychological stressors, i.e., social approach (animal)
Startle-based paradigms	Startle
	Fear-potentiated startle
Challenge-induced panic	CO_2 challenge
	Yohimbine challenge
	Lactate challenge (human, animal); PAG stimulation (animal)
	Corticotropin releasing factor (CRF) challenge
	Cholecystokinin-tetrapeptide (CCK-4) challenge
Conflict response	Conflict models, i.e., Vogel, Geller-Seifter
Physiological arousal	HPA-axis activity
	Autonomic arousal (HR, BP, skin conductance, body temp)

The potential role of glutamate in anxiety disorders was initially recognized in preclinical paradigms using ionotropic glutamate receptor antagonists acting at NMDA or AMPA receptors (for review, see Bergink et al. 2004). Glutamate is the major excitatory neurotransmitter in the brain, with widespread projections and localization of its various receptors. It is involved in numerous functions, not only the mediation of anxiety, but also in pain perception and cognition. Activating or deactivating a receptor for such a pervasive neurotransmitter has the potential to produce such serious side effects that they render NMDA and AMPA receptors difficult targets for drug development. For example, blockade of NMDA receptors may not only produce anxiolytic effects but also risks impairment of cognitive function and producing schizophrenia-like symptoms. Memantine, an uncompetitive NMDA antagonist with moderate affinity, is currently approved for treatment of moderate to severe Alzheimer's disease, pointing to the potential for glutamate antagonism as a drug treatment if alterations are in moderation (Parsons et al. 2007). Researchers have turned to the metabotropic glutamate receptors to more subtly alter glutamate transmission, and preclinical data seem to support the concept that manipulation of at least some metabotropic glutamate receptor subtypes has anxiolytic-like effects with a relatively low incidence of unwanted side effects (Spooren et al. 2001, 2003).

The metabotropic glutamate receptors are a novel class of receptors under investigation as a new target for multiple therapeutic indications, including anxiety. The eight identified metabotropic glutamate receptors are further divided into three classes based on sequence similarities, pharmacological and biochemical properties: Group I ($mGlu_1$ and $mGlu_5$), group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$). The extensive literature showing anxiolytic-like effects of manipulation of metabotropic glutamate receptors in various preclinical models has been reviewed elsewhere (Spooren et al. 2001, 2003; Spooren and Gasparini 2004; Swanson et al. 2005), and will therefore not be the focus of this review. Rather, this review will emphasize the important role that translational research has played in understanding of the potential for metabotropic glutamate receptor modulation in anxiety,

identify lacunas where animal and human research have yet to be connected, and suggest areas where translational research can be further developed.

Metabotropic glutamate receptors in translation

Despite the large amount of research that has accumulated supporting the potential role of metabotropic glutamate receptors from class I and II, including work in potentially translational paradigms in rodents, there is a relative paucity of research in which human and animal results can be directly compared. The clearest examples of work which can be compared derive from studies on pharmacodynamic markers, investigating whether a compound interacts appropriately with its molecular target at its target organ, i.e., a receptor in the brain. For example, a PET ligand for the $mGlu_5$ receptor has been developed, allowing a direct comparison of the level of receptor occupancy and distribution of $mGlu_5$ agonists or antagonists in animals and humans in vivo (Ametamey et al. 2006, 2007; Wyss et al. 2007). The development of surrogate efficacy biomarkers has been more challenging, although the effects of the $mGlu_2$ agonist LY354740 in fear-potentiated startle in both animals and healthy volunteers have been described and can serve as an example for such an approach (Bueno et al. 2005; Grillon et al. 2003; Helton et al. 1998; Johnson et al. 2003; Tizzano et al. 2002; Walker et al. 2002). This and $mGlu_5$-related PET ligand research will subsequently be described, followed by examples of studies in which direct comparisons are more difficult and a discussion of research in "translatable" paradigms that so far has been conducted in either humans or animals, but not both.

Translational research with mGluR binding: development of a PET ligand

The question of potential species differences in target receptor distribution or in binding profiles of a compound is ever-present in drug development. Recent developments in imaging technology, specifically increased resolution, allow imaging in rodents and non-human primates alongside human studies and can aid in comparing distributions across species. In a series of studies testing the selective $mGlu_5$ PET ligand ^{11}C-ABP688 in rats, humans and mice, Ametamey et al. were able to demonstrate very similar patterns of ligand binding in these species (Ametamey et al. 2006, 2007; Wyss et al. 2007). These studies showed high binding in the hippocampus, striatum, and cortex, in accordance with distribution patterns seen using immunohistochemistry and in situ hybridization for the $mGlu_5$ receptor in both human (Daggett et al. 1995) and rat brain (Romano et al. 1995). Specificity of the PET ligand was confirmed by the lack of binding in $mGlu_5$ knock out mice (Ametamey et al. 2006).

Aside from its obvious utility for comparative anatomy of the $mGlu_5$ receptor distribution in vivo, having an $mGlu_5$ receptor ligand such as ^{11}C-ABP688 also presents the possibility to conduct blocking and competition studies in vivo similarly to work that has been conducted on other receptors, i.e., NMDA (Ametamey et al. 1999), benzodiazepine (Bottlaender et al. 1994; Lingford-Hughes et al. 2002), and dopamine (Kassiou et al. 2002; Nikolaus et al. 2005) receptors. By circumventing the need to radiolabel each novel compound, competition and blocking studies can considerably speed imaging studies with new compounds. The difficulty in developing a reliable radiotracer is perhaps best illustrated by the numerous failures along the road to developing a tracer for the $mGlu_5$ receptor. For example, despite the clear value of the reference standard $mGlu_5$ antagonist MPEP in behavioral work and the suitability of [^{3}H]MPEP for ex vivo receptor occupancy studies (Steckler et al. 2005b), [^{11}C]MPEPy has thus far proven to be an inadequate tracer for imaging when tested in non-human primate (Severance et al. 2006). It is important to bear in mind, however, that mGlu receptors can be pharmacologically influenced by compounds binding either allosterically or orthosterically (Kew and Kemp 2005). ^{11}C-ABP688 is an allosteric antagonist of the $mGlu_5$ receptor, meaning that its usefulness in determining binding profiles for orthosterically binding compounds may be limited.

A caveat in the translational potential for imaging studies is that most rodent studies are performed in animals under anesthesia to provide sufficient immobilization, thus precluding imaging while performing tasks and essentially limiting the use of imaging in rodents to occupancy studies. There have, however, been a number of efforts to image the brain in awake rodents, with both fMRI and (micro)PET (Hosoi et al. 2005; Lahti et al. 1998; Momosaki et al. 2004), and in alert monkeys with fMRI (Logothetis et al. 1999). Thus far, only passive reactivity to, for instance, whisker or forepaw stimulation (Peeters et al. 2001), hypoxia (Duong 2007), or pharmacological stimuli (Chin et al. 2006; Skoubis et al. 2006) has been measured in rodents. The ability to image awake animals raises the possibility of testing reactivity to stimuli under the influence of a test compound and to relate brain activity to behavior. For example, one could measure reactivity to conditioned stimuli in a classical conditioning paradigm, such as a tone, which would certainly bear relevance in research on anxiolytics, although the need to restrain animals during imaging and the potential stress this may bring confound results and would need to be carefully controlled.

Another potential hurdle for the use of imaging in small rodents is that of resolution. While spatial and temporal

resolution are improving at a rapid rate in this relatively young technology, with recent reports showing sub-millimeter resolution in fMRI signals (Moon et al. 2007), at present, both temporal and spatial resolution for PET are sub-optimal. A possible complementary approach to facilitate higher temporal resolution of radioligand binding is the newly developed β^+-sensitive microprobe (Pain et al. 2002). This microprobe measures radioactivity, and has been used with 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG), a commonly used tracer for glucose used as an indirect measure of brain activity, and [^{11}C]raclopride, commonly used to measure dopamine D_2 receptor and receptor occupancy levels (Mauger et al. 2005). This technique has also been used successfully to measure levels of the $mGlu_5$ PET ligand discussed above, ^{11}C-ABP688 (Wyss et al. 2007). Thus far, only data in anesthetized rats have been published. However, this technique is potentially feasible to be performed in freely moving animals, as the microprobe is implanted similarly to microdialysis probes.

Translational research in $mGlu_{2/3}$ efficacy: fear-potentiated startle

The fear-potentiated startle paradigm is strongly represented in anxiolytic research conducted in animals, and was recently adapted to a version for human research that is suitable for pharmacological testing (Grillon and Baas 2003; Risbrough and Stein 2006). The basic procedure is represented schematically in Fig. 1. In this test, the response is measured to a startling stimulus, such as a loud noise, either alone or during a "threat" situation. The "threat" situation is usually a light which signals that an aversive stimulus, such as a mild electric shock, may occur. Normally, the startle response is potentiated during the "threat" situation. This method had been in use in animal research for some time, using the efficacy of anxiolytics in the paradigm to justify its use in anxiolytic research. Grillon et al. have conducted a series of studies validating the paradigm by demonstrating increased fear-potentiated startle in patients with post-traumatic stress disorder (Grillon et al. 1998b, c; Morgan et al. 1995), panic disorder (Grillon et al. 1994), and children of parents with panic disorder (Grillon et al. 1998a). This is a case where "bench-to-bedside" translation has provided useful information for preclinical researchers.

The finding that the $mGlu_{2/3}$ receptor agonist LY354740 reduces fear-potentiated startle in humans (Grillon et al. 2003), as it does in rats (Helton et al. 1998; Johnson et al. 2003; Tizzano et al. 2002), has been a major step forward in translational research in the field of anxiolytics (Fig. 2). However, LY354740 is relatively poor brain penetrant (Rorick-Kehn et al. 2007), raising the question of the locus of action in studies involving systemic drug administration.

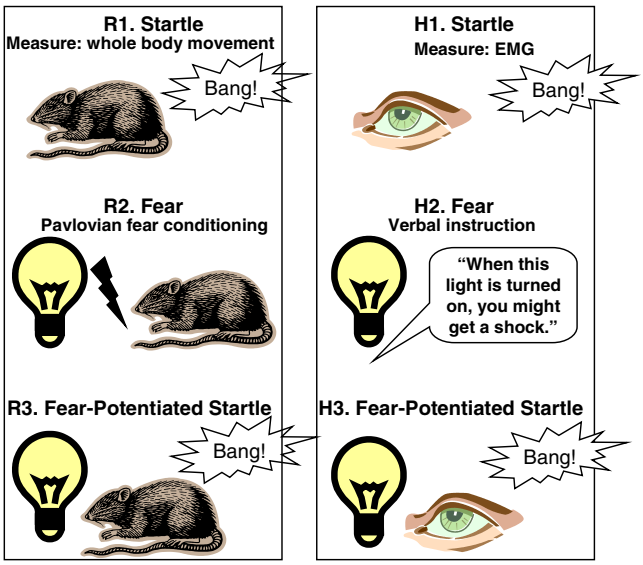

Fig. 1 Examples of protocols used to test fear-potentiated startle in humans and in rats. In the left panel, the rat protocol is illustrated. In the right panel, the human protocol is shown. Briefly, startle responses in the form of whole-body movement (*rat*) or EMG (*human*) are measured in the presence or absence of a fear-eliciting stimulus. In rats, startle responses are measured as velocity of movement in response to a loud noise (*R1*). Fear of a specific stimulus is induced by Pavlovian fear conditioning, in which a footshock is delivered simultaneously with the illumination of a light, inducing a fear response to the light (*R2*). Fear-potentiated startle is elicited by exposing the rat to the same loud noise from *R1* in the presence of the light used as a conditioned stimulus from *R2* and measuring the whole-body startle response, shown in *R3*. Fear-potentiated startle is defined as the startle measured in panel *R3*, minus the startle measured in *R1*. The human protocol is very similar: startle to a loud noise is measured by EMG measures of the eyeblink reflex (*H1*). Fear is coupled to a light stimulus by instructing the subjects that when they may receive a shock when the light is illuminated (*H2*). Fear-potentiated startle is elicited, as in rat, by exposing subjects to a loud noise in the presence of the fear-coupled light (*H3*), with fear-potentiated startle defined as the eyeblink response EMG measured in *H3* minus the eyeblink response EMG measured in *H1*

Regardless, it is interesting that in contrast to the results found with LY354740, benzodiazepines have shown mixed results in the fear-potentiated startle paradigm in humans (Baas et al. 2002; Bitsios et al. 1999; Riba et al. 2001; Scaife et al. 2005, 2007), with strong evidence that benzodiazepines affect associative learning aspects of the paradigm rather than having a selective effect on fear potentiation. In a study in which LY354740 and the benzodiazepine diazepam were compared in rats, results paralleled human studies with LY354740, selectively affecting fear-potentiated startle but diazepam reducing both fear-conditioning and fear-potentiated startle (Tizzano et al. 2002).

The examination of human subjects in a paradigm originally developed for animals has built a bridge between

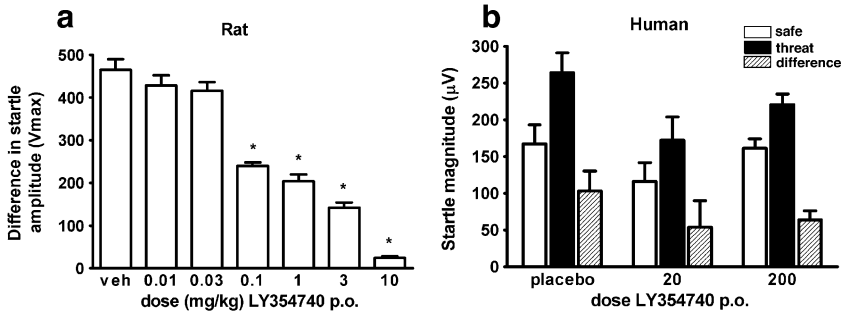

Fig. 2 Sample data showing suppression of fear-potentiated startle by LY354740 in rat (**a**) and human (**b**). A seen in panel **a**, LY354740 dose-dependently decreases startle responses in rat, a decrease that is significant from 0.3 mg/kg. In human studies, the effects are more subtle, with post-hoc testing showing a significant difference between placebo vs. drug treated groups (thus 20 and 200 mg combined) in the "difference" condition that represents fear-potentiated startle. Rat data is modified from Helton et al. (1998); human data is modified from Grillon et al. (2003). Figures reproduced with permission

ongoing clinical studies on LY354740 and a large body of animal research concerning this compound. An important aspect to keep in mind, though, is the question of the validity of the fear-potentiated startle model in measuring anxiety. The question arises whether startle measures fear or surprise, representatives of two separate psychological constructs. Nonetheless, the use of fear-potentiated startle in both animals and humans is certainly an example worth following up, first by confirmation in other laboratories to ensure reproducibility of fear-potentiated startle in humans, and then both by assessing this compound in other "translatable" models and by testing other compounds in the human fear-potentiated startle paradigm.

Different methods to model the same symptom: $mGlu_{2/3}$ in sodium lactate-, CO_2-, and CCK-4- induced panic

A panic attack is a sudden burst of severe anxiety accompanied by physical symptoms including cardiorespiratory and autonomic symptoms, which can occur as the defining symptom in the context of panic disorder (for review, see Roy-Byrne et al. 2006), or as a comorbid symptom in patients with other disorders including generalized anxiety disorder, social phobias, depression, bipolar disorder, and obsessive–compulsive disorder (Angst et al. 2005; Goodwin and Gotlib 2004; MacKinnon and Zamoiski 2006; Nutt et al. 2006). A number of methods have been employed to induce panic attacks experimentally in humans and animals, either by injection or inhalation of panicogenic substances, such as CO_2, yohimbine, lactate, corticotrophin-releasing factor or cholecystokinin-tetrapeptide (CCK-4). Some of these methods work exclusively in panic-prone individuals, while others also produce panic-like reactions in healthy controls. As discussed below, LY354740 has been tested in several panic models and has demonstrated reductions in panic and panic-like reactions in animals. It is noteworthy that, although LY354740 has been tested in both animals and humans in evoked panic paradigms, the drug has yet to be tested in the same panic paradigm in animals and humans, a serious impediment to translation of the results. One reason for this discrepancy could be the difficulty to back-translate some of the human data to reliable animal models.

Panic can be induced in panic-prone humans by infusions of sodium lactate (Maddock 2001), or by inhalation of high concentrations of CO_2 (Battaglia and Ogliari 2005; Rassovsky and Kushner 2003; Zvolensky and Eifert 2001). These paradigms also produce panic-like symptoms in animals that have been made more sensitive to the treatment, for instance by chronic inhibition of GABA synthesis by infusion of L-allyglycine in the dorsomedial hypothalamus, a treatment which causes increased heart rate, blood pressure and social interaction on sodium lactate infusion (Sajdyk and Shekhar 2000; Shekhar et al. 1996). However, the induction of a *reliable* anxiogenic-like response by CO_2 in otherwise unchallenged rodents seems to be much more difficult (Pouzet and Steckler, unpublished data). Sodium lactate is metabolized to bicarbonate, and then to CO_2 when it crosses the blood–brain barrier, thus it has been suggested that underlying mechanisms in the CO_2 and sodium lactate paradigms may be similar (Battaglia and Ogliari 2005), although this explanation has been debated (Klein 1993). It has been proposed that the induction of panic by CO_2 is not only a useful research tool but that CO_2 inhalation may resemble the actual origin of panic attacks, which are hypothesized to be a false alarm signal of suffocation (Klein 1993, 1994). While this interpretation is still controversial (Smitherman 2005), nonetheless, CO_2 inhalation has proven to be sensitive to various anxiolytics and a useful paradigm for testing agents in alleviating panic. The $mGlu_{2/3}$ agonist LY354740 has been shown to be effective in reducing panic-like cardiovascular responses induced by infusion of sodium lactate in panic-prone rats (Shekhar and Keim

2000). The compound was reported in an abstract to be effective in reducing panic following CO_2 inhalation in panic disorder patients (Levine et al. 2002). The similar results in analogous paradigms in panic-prone rats and humans is encouraging, but as CO_2 inhalation has been reported by some authors to provoke anxiety-like symptoms in rats (Battaglia and Ogliari 2005), and sodium lactate infusion provokes panic attacks in panic-prone patients (Maddock 2001), it would be helpful if a single paradigm was used to facilitate direct comparison.

In contrast to CO_2 or lactate-induced panic, CCK-4 can cause panic in healthy subjects (Jerabek et al. 1999a, b) as well as in normal animals (van Megen et al. 1996), although again a *reliable* anxiogenic-like effect of CCK-4 in normal rodents has not been observed in all studies (Pouzet and Steckler, unpublished data). CCK-4-induced panic may also have a different neurobiological basis (Bradwejn and Koszycki 2001) compared to CO_2 or lactate-induced panic. Oddly, CCK-4 and CO_2 panic are not addictive, but pretreatment with CCK-4 actually lessens susceptibility to CO_2-induced panic in healthy volunteers (Schruers et al. 2000), which may raise questions about the validity of these methods. The CCK system may be disturbed in patients with panic disorder, as the panic response to CCK-4 is enhanced in panic disorder patients and there have been reports of possible genetic links between panic disorder and the alterations in the CCKB receptor (Hösing et al. 2004; Kennedy et al. 1999). In a recent study in healthy human volunteers, LY544344 (the prodrug of LY354740) reduced CCK-4-induced panic (Kellner et al. 2005), an indication that this drug may be useful as an anti-panic agent (Steckler 2007).

Thus, while LY354740 has been tested in humans in the CO_2 and CCK-4-induced panic paradigms, and in rats in lactate-induced panic, and all show anxiogenic properties, there is no direct overlap between test methods in animals and humans. The demonstrated efficacy of LY354740 in different paradigms strengthens the case for the use of LY354740 in anxiety; indeed, the drug was recently shown to be effective in treating generalized anxiety disorder (Dunayevich et al. 2008). However, in terms of translational research, it would be illustrative to compare the drug in congruent paradigms in humans and rodents. This would facilitate efforts to validate translational research, by promoting better understanding of the importance (or unimportance) of using the same paradigms in animals and humans in drug development. Coordinated efforts between facilities testing humans and animals are needed to align animal and human research at an early phase and allow direct comparisons between animal and human data, for instance, by using paradigms taxing comparable brain mechanisms and assessing comparable outcome measures in animals and humans.

Preclinical methods with translational merit: stress-induced hyperthermia, conflict behavior and HPA-axis measures

Stress-induced hyperthermia is an example of an underexploited but potentially translatable model of physiological responses to stress, which can be conducted in both animals and human subjects with minimal interventions. Mice react to a mild stressor, such as combined handling and insertion of a rectal thermometer, with a mild hyperthermia in which core temperature increases approximately 0.5°C to 0.8°C. This response has been demonstrated to be sensitive to anxiolytic drugs from various classes (Bouwknecht et al. 2007). Stress-induced hyperthermia was also demonstrated to retain its magnitude of effect after repeated trials in mice, rendering the method acceptable for testing in chronic situations (Nordquist et al. 2007). Humans also respond to stress with hyperthermia, for instance, to a stressor in the form of university exams (Briese 1995; Marazziti et al. 1992). $mGlu_1$ receptor antagonists (Rorick-Kehn et al. 2005), $mGlu_{2/3}$ receptor agonists (Johnson et al. 2005; Rorick-Kehn et al. 2005, 2006; Spooren et al. 2002), and $mGlu_5$ receptor antagonists (Nordquist et al. 2007; Rorick-Kehn et al. 2005; Spooren et al. 2002) have been demonstrated to reduce the magnitude of stress-induced hyperthermia in mice. Given the relative simplicity of the model and the solid evidence from mouse studies that modulation of metabotropic glutamate receptors play a key role in stress-induced hyperthermia, this method would be a candidate to further investigate the role of metabotropic glutamate receptors in anxiety in humans.

In so-called conflict paradigms, subjects are permitted to perform a response for a positive reinforcer. The response differs in various paradigms; this could be an operant response, such as lever pressing for food or pressing a button to see art, or a consummatory response, such as drinking water. While the subjects are performing this response, either an aversive stimulus (such as a shock) or a stimulus which was previously classically conditioned to an aversive stimulus is presented, which normally causes a decrease in responding for the positive reinforcer (originally described in rats in Gellert and Seifter 1960; more recent data in Barros and Tomaz 2002; Fuchs and Flügge 2006; Millan and Brocco 2003). This decrease in responding is sensitive to anxiolytic as well as some antidepressant drugs in rodents (Borsini et al. 2002) and non-human primates (Hanson et al. 1967; Sepinwall et al. 1978). Among the anxiolytics shown to be effective in attenuating a conflict-induced decrease in responding are compounds which bind to type I or type II metabotropic glutamate receptors (Ballard et al. 2005; Busse et al. 2004; Klodzinska et al. 2004; Pietraszek et al. 2005; Pilc et al. 2002; Stachowicz et al. 2007; Steckler et al. 2005a; Varty et al. 2005). Conflict models have long been demonstrated to be effective in humans (Di Giusto and

Bond 1978; Nelson and del Carmen Sanjuan 2006; Salgado et al. 2000); however, they have been used only in a small-scale study in human research with the anxiolytic diazepam (Beer et al. 1975), though this study did show promising results. Because of the large body of research covering anxiolytic action in conflict models in rodents, and the long history of use of this model in humans, this could be another interesting approach to explore for translational use.

Patients with anxiety also present abnormalities in regulation of the hypothalamic-pituitary-adrenal (HPA) axis, with reports of blunted ACTH response to CRF (Curtis et al. 1997; Holsboer et al. 1987; Roy-Byrne et al. 1986) indicative of a possible increase in negative feedback within the HPA axis in patients and abnormal circadian rhythms in cortisol secretion (Abelson and Curtis 1996). Inhalation of 35% CO_2, a model for panic discussed above, also causes increased HPA responsivity in the form of enhanced cortisol and ACTH levels in both panic patients and control subjects (van Duinen et al. 2005, 2006). In human healthy volunteers, exposure to psychosocial stressors in the Trier Social Stress Test, involving a mock job interview and public speaking, induces HPA activity (Ising et al. 2007; Kudielka et al. 2004a, b). Given the relative ease with which HPA responsivity can be measured in animals, this would seem to be another highly translatable model.

However, the case of HPA reactivity may be more complex than it first seems. In a recent test of CCK-4 induced panic, ACTH was found to be raised by CCK-4 regardless of whether or not a panic attack actually occurred (Eser et al. 2007). Conversely, sodium-lactate-induced panic does not coincide with an increased ACTH response in anxiety disorder patients or control subjects (Otte et al. 2002) and an anxiogenic effect but no HPA-axis activation was reported in a recent poster from Bailey et al. following exposure to 7.5% CO_2 concentrations (Bailey et al. 2007). Furthermore, a number of (putative) anxiolytic drugs, including buspirone, some benzodiazepines (De Boer et al. 1990, 1991a, b; Matheson et al. 1996), and mGlu receptor agonists/antagonists (Bradbury et al. 2003; Johnson et al. 2001) actually raise HPA-axis measures upon first administration, and there are conflicting reports on whether anxiolytics raise, lower, or attenuate HPA reactivity after repeated dosing (Jezova 2005). Thus, although HPA reactivity may be an easily translatable measure, the interpretation of the measure in the context of anxiety is unclear and thus, this measure should be approached with caution.

General questions in translational medicine: what is translatable?

Thus far, this review has concentrated on issues of translation specifically related to development of anxiolytic drugs. Alongside the field-specific issues, there are also issues which touch research in all disease areas, not just anxiety or indeed central nervous system disorders. Collaboration between disease areas, and with safety and toxicology departments as well, are of great benefit to address some of the issues that will be discussed in the following section.

Biomarkers: physiological measures of psychological disposition?

A biomarker has been defined as "a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process, or pharmacologic response to a therapeutic intervention" (Atkinson et al. 2001). An objective measure in clinical studies of psychiatric disorders, which are currently dependent upon subjective measures such as rating scales to judge efficacy of interventions, would clearly be beneficial. Indeed, regulatory agencies, including the FDA, are warming to the prospect of including biomarkers in regulatory submissions (Mendrick 2007), further incentive to both industry and academia to investigate possibilities to use biomarkers both for predicting individual reactivity to specific drugs and monitoring outcomes. A three-pronged classification system of biomarkers has been proposed (Mildvan et al. 1997) in which markers are classified as natural history markers (type 0) that reflect underlying pathogenetic mechanisms and can predict clinical outcome with or without treatment; biological activity markers (type 1), which respond to therapy; and surrogate markers of therapeutic efficacy (type II), a single marker or composite of several markers that can fully account for the efficacy of an agent. It has further been suggested that type II biomarkers could eventually be surrogates for endpoints in clinical trials (Atkinson et al. 2001; Mildvan et al. 1997). The concept of surrogate endpoints was made in reference to illnesses with a more circumscribed somatic basis than psychiatric diseases, such as HIV and diabetes, and absolute replacement for measures of subjective aspects like affect may be unattainable in psychiatric research. This does not, however, diminish the usefulness of biological activity markers in measuring responses to compounds in tandem with rating scales and clinical observations.

In terms of translation, both natural history markers and biological activity markers are aspects which can be developed in animals and translated to humans. Natural history markers can entail genetic testing, or measure of protein or hormone levels. In terms of genetic testing, recent developments in quantitative trait loci (QTL) analysis may prove useful for identification of genes that predispose individuals to anxiety or predict response to anxiolytics (Letwin et al. 2006; Singer et al. 2005). The

technique of QTL has the distinct advantage that it can, in principle, function as an open screening method, where the starting point is a phenotype and the end result, a marker related to this phenotype. This is in contrast to pharmacological studies which use a predetermined target or set of targets (receptor, transporter, or other mechanism) and investigate these targets in a specific paradigm, which by definition, limits the targets investigated to known targets. The potential usefulness of QTL was demonstrated in a recent linkage study in humans, which showed linkage between the PLXNA2 gene and anxiety in humans, while the mouse ortholog has also been reported as a locus related to anxiety-like behaviors (Wray et al. 2007). Resolution of QTL analysis has been a consistent problem, with loci identified to chromosomes but much too large to identify genes. Advances are being made in analysis techniques that allow finer resolution of QTL (Valdar et al. 2006). It should be noted, however, that in the study by Valdar et al. using analysis techniques allowing finer resolution of loci, of 17 physiological, immunological, and anxiety-related traits studied in more than 2,000 mice, QTL were found for all traits except four, three of which were anxiety-related. This lack of loci for trait anxiety in such a large study emphasizes the difficulty of finding a genetic link to anxiety, and the multifactorial nature of anxiety disorders.

At the level of proteins and hormones, biomarkers can be of either type 0, indicating predisposition, or type 1, which alter in response to treatment. In the field of anxiety, efforts have been primarily focused on HPA-related hormones, indicating alterations in steady-state conditions, stress reactivity, and alterations following drug treatment (see "HPA-axis measures" section above). However, as discussed above, HPA activity is difficult to interpret because, by their very nature, HPA-related responses are highly sensitive to stressors. This conflict may be difficult to resolve, as it is likely that most proteins or hormones related to anxiety will also be related to stress.

Allometric scaling and determination of doses

Aside from having translatable tests at hand that allow one to bridge preclinical and clinical pharmacology from a conceptual point of view, it is also important to understand the relationships between the dose of a compound administered and the time-course of the plasma and brain exposure following administration of such compound across preclinical species to accurately predict key human pharmacokinetic parameters. Prediction and subsequent validation of plasma pharmacokinetics is relatively easy in humans, as plasma is a common matrix for drug concentration measurements in human. Validation of brain pharmacokinetics in human is virtually impossible. The assumption is that for brain levels, if the plasma and brain PK are understood from the pre-clinical species, then the same relationship could be occurring in human. Extrapolation of plasma pharmacokinetic results from animals to human considers the interspecies relationships between clearance (hepatic and or renal blood flow) or volume of distribution of (unbound) drug and species body weight (Mahmood et al. 2003). The relationships for these are studied in the preclinical species and then extrapolated to humans (Obach et al. 1997). This allows for the prediction of two of the key human PK parameters, clearance and volume of distribution, and then the estimation of a third, half life ($t1/2$; Fig. 3). The purpose of predicting human half life is to assess potential dosing regimens (once a day, twice a day, etc.). To provide support for this assumption and to perform allometric scaling, the plasma pharmacokinetics, plasma protein binding and the in vitro metabolic clearance from three pre-clinical species is required, plus the brain pharmacokinetics over time for example in the pharmacodynamic species. Such pharmacokinetic information can then be integrated with preclinical pharmacodynamic measures, e.g., with information about concentration–response relationships in relevant anxiety tests and target receptor

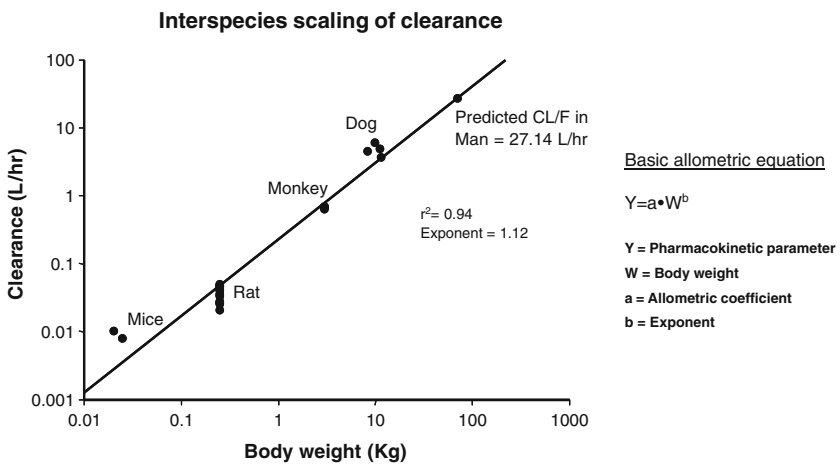

Fig. 3 Example of prediction of human clearance using simple allometry; modified from Sinha et al. 2008

occupancy (in vivo) as a function of concentration and time. These scaled pharmacokinetics, together with pharmacokinetic and pharmacodynamic relationships and therapeutic index, in turn, allow one to identify an optimal dosing regime for proof of concept studies in humans (Peck et al. 1994).

A number of different allometric scaling approaches exist that guide the prediction of human pharmacokinetics and estimations of a starting dose in humans based on such animal data (Boxenbaum 1982; Boxenbaum and DiLea 1995; Mahmood and Balian 1996; Obach et al. 1997) and are routinely employed within the drug development process. The value of this approach in predicting the major human PK parameters of clearance and volume of distribution can be illustrated by a recent publication on the Group II metabotropic glutamate receptor agonist MGS0028 (James et al. 2005). To characterize the suitability of this compound for clinical development regarding the PK and to select the most appropriate preclinical species to use for further development of the drug with respect to toxicology species, the in vitro and in vivo metabolic and disposition profile was assessed in three preclinical species (rat, dog, and monkey) and compared to in vitro data using human cellular assays. This led to two important conclusions: first, that rats and monkeys would be the species of choice for preclinical development of the compound prior to testing in humans. Secondly, allometric scaling of the compound predicted that the major human pharmacokinetic parameters (clearance and volume of distribution) would be acceptable for further development of the compound (James et al. 2005). Over time, the prediction of human oral pharmacokinetics from preclinical species has become of great interest as the oral route is the desired route of administration in the clinic. Until now, little has been published on this possibly due to the added complexity of absorption and gut wall metabolism which doesn't have to be taken into account for the empirical allometric approach for intravenous data scaling. More recently, publications have evaluated approaches for the allometric scaling of oral clearance (Feng et al. 2000, Mahmood 2002, Tang and Mayersohn 2005). This has been further evaluated by Sinha et al. (2008), where the performance of four different allometric approaches for the prediction of oral clearance and oral area under the curve has been discussed.

Effect size and translational medicine

One of the principal advantages of animal research is the ability to strictly control experimental conditions and to use inbred strains of rodents for testing in order to minimize within-group variability. Despite the best efforts to control experimental conditions and to use homogenous populations, clearly, the variability in human research will always be larger than in animal research. This has important implications for many of the paradigms discussed in the present manuscript. For instance, stress-induced hyperthermia involves a body temperature increase of 0.5°C–0.8°C, an increase which can also fairly easily be caused by other mild hyperthermic processes such as exercise. This increase can be detected in humans (Marazziti et al. 1992), and pharmacological changes in stress-induced hyperthermia can be detected under strictly controlled conditions in animals (Bouwknecht et al. 2007; Spooren et al. 2002), however, it remains to be seen if pharmacological interventions will cause alterations in stress-induced hyperthermia with a small enough variability to be statistically reliably detected. Similar warnings may apply to fear-potentiated startle, which produces relatively small effects in humans. For these tasks, and possibly others designed for animals and translated to humans, the potential for false negatives in human testing needs to be recognized, and thus, negative results in humans in these tasks should be viewed in light of results from other paradigms and animal results.

The underlying rationale behind translational models in drug development is usually an attempt to circumvent long development trajectories using animal studies that have limits in terms of predicting efficacy or doses in humans. From small studies with human subjects, one would hope to draw conclusions regarding the potential of a drug for treatment of humans with a specific indication. To be truly useful in drug development, such experiments need to be able to provide clear go/no go advice for clinical testing. At present, models which have been adapted from animal research to human research are consistently in danger of having too little power, as discussed above. On the one hand, this could mean unnecessary rejection of potentially effective compounds. However, one of the reasons for the long course of drug development, and for the development of translational medicine, is the number of false positives that animal preclinical studies produce (Perel et al. 2007; Unger 2007). Keeping only the compounds with efficacy in human studies, a relatively stringent selection criteria may be one way to weed out false positives early in the development process. In that sense, relatively low power may not be a disadvantage, but rather a strength to translational medicine approaches.

Conclusions: status of translational research

The general concept of translational medicine is not novel, as the basic premise of animal research is that it can be translated into useful information for humans. However, recent efforts to align animal and human research will

certainly facilitate this translation and as such should be encouraged. In this review, we have exemplified the potential for translational research to aid drug development with research conducted in anxiety using metabotropic glutamate receptor modulators. We highlighted the value of cross-species use of imaging and the usefulness of translating fear-potentiated startle, a paradigm familiar to animal researchers, into human research. As a whole, this body of research indicates translatability of metabotropic glutamate receptor anxiolytic properties, strengthening arguments for development of this class of drugs as anxiolytics. Stress-induced hyperthermia, HPA-axis measures, and CO_2-, lactate-, or CCK-4-induced panic are cited as examples of translatable paradigms that need alignment between animal and human experimentation. Finally, the potential for translational medicine which cut across various fields of research, such as with biomarkers and allometric scaling, are crossroads where different fields meet and cross-disciplinary cooperation can bear fruitful results. The caveats discussed, including the small size of rodent brains compared to resolution of imaging systems and the potential for relatively small effects to become lost in translation, need not preclude translational research, but should be taken into account in interpretation. Alignment between animal experimentation, human research, and clinical drug development can only take place if clinical and preclinical departments work together more closely with a thorough understanding of the advantages and limitations of translational research.

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