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Report Targeted Expert Meeting – Anxiety Disorders and Anxiolytics 11-12 September 2009, Istanbul, Turkey

Coordinator: Astrid Linthorst, United Kingdom
Co-chair: David Baldwin, United Kingdom

I. Summaries of lectures and discussions

Topic 1 – The role of glutamate in the pathophysiology and treatment of anxiety

Main Lecturer: Dai Stephens, United Kingdom
Glutamate, the brain's major excitatory transmitter, acts via both ionotropic and metabotropic receptors. Glutamate plays an essential part in neural systems underlying emotions, including fear and anxiety, making its receptors plausible targets for novel anxiolytic agents. Issues to be solved include which receptor subtypes, and which anxiety disorders to target. The wide role of glutamate in neurotransmission poses issues of specificity for the targeted disorder of any drug based on glutamatergic transmission, and side-effects are likely to be problematic.
Antagonism of glutamatergic NMDA ionotropic receptors by competitive and non-competitive antagonists has reliable anxiolytic-like effects in animal models, though clinical data are limited. Very limited clinical data on memantine, that may possess a less unfavourable side effect profile, suggest a possible efficacy in posttraumatic stress disorder (PTSD), and perhaps obsessive compulsive disorder (OCD), but not in generalized anxiety disorder (GAD). There are only limited preclinical and no clinical data supporting use of AMPA-R antagonists in anxiety. In PTSD there are early positive data to suggest kainate receptors as potential targets and larger scale clinical studies may be warranted.
Antagonists of mGluR5 metabotropic receptors are active in animal models, and one older clinically active anxiolytic, fenobam, with a previously unidentified mechanism of action, is now known to act in this way. Reducing glutamate release by activating presynaptic mGluR2/3 receptors might offer an alternative strategy, and one such compound is active preclinically, in human experimental medicine models, and initial clinical trials of GAD, but not panic disorder (PD).
A novel approach that has already been applied experimentally and found useful in treating PTSD or specific phobias, is to use partial agonists at NMDA receptors to facilitate extinction of fear memories.

Discussions
The first discussant, Sanjay Mathew (USA) reported on clinical studies on the anxiolytic potential of two drugs that target the imbalance in glutamate homeostasis. Riluzole, approved for the treatment of amyotrophic lateral sclerosis (ALS), is a glutamate vesicular release inhibitor and plasticity enhancing agent. However, it has various other effects including increased re-uptake of glutamate in astrocytes. As such riluzole may assist in decreasing a potentially hyperactive glutamate system in anxious patients. It was found that riluzole is indeed therapeutically effective in patients with GAD and there was a positive correlation between a therapeutic response to riluzole and its effects on hippocampal levels of NAA, a potential index for glutamate neurotransmission and turnover, as measured by ^{1}H MRS. Next, data were presented on the effects of intravenous infusion of the NMDA receptor antagonist ketamine on

anxiety measured in a group of treatment-resistant depressed patients. Subanaesthetic doses of ketamine reduced the severity of depressive symptoms significantly and some indications for reduced anxiety, e.g. improvement in inner tension rating on the MADRS scale; decreased CAPS (Clinician-Administered PTSD Scale) scores in two patients with co-morbid diagnosis of PTSD, were also found; however, all patients had relapsed one week after treatment. Various relapse prevention strategies are currently being tested although it was found in a small sample size that riluzole was not effective in this respect. These data clearly demonstrate the potential of glutamatergic drugs in the treatment of anxiety but also the need for more research especially with respect to the robustness of remission.

Next, Thomas Steckler (Belgium) discussed the development of anxiolytic glutamatergic drugs from a drug development point of view. While he concluded that preclinical and clinical data suggest that the glutamatergic approach to treating anxiety disorders holds promise, the evidence that these new drugs are more efficacious, and faster in onset, than the currently used drugs is still outstanding. Furthermore, it would be important to reduce the side effects as some of the glutamatergic compounds exerted negative effects in animal models on locomotor function, social interaction and cognition, and had psychotomimetic effects. Interestingly, he argued that the results obtained with mGlu1 and mGlu5 positive allosteric modulators may lead to a paradigm shift, i.e. they may show promnestic effects and increase fear extinction; observations which may be of interest for the further development of drugs for the treatment of PTSD.

During the plenary discussion after this topic several aspects were covered in detail. It was felt that glutamatergic compounds hold promise. However, there were serious concerns about the side effects, especially with respect to memory. The participants felt that it is crucial to increase our knowledge on:

- the underlying neurobiological mechanisms, especially on the role of glutamate receptors in different brain regions: there is a brain region selectivity in expression profiles
- the precise neuronal localisation: pre- versus postsynaptic,
- the role of receptors expressed in glial cells.

It was further recognised that development of allosteric modulators would be the most promising way forward as such modulators, in contrast to orthosteric ligands, would only exert their action in activated glutamatergic neuronal circuits. The discussion on which specific anxiety disorders glutamatergic compounds should focus led to a more general discussion on how animal models of anxiety relate to the human disease situation.

It was concluded that the translational aspects of research into anxiety disorders should be improved both at the preclinical (animal models for fear versus anxiety) and at the clinical (classification of anxiety disorders) side.

Topic 2 – The role of endocannabinoids in the pathophysiology and treatment of anxiety

Main Lecturer: Beat Lutz, Germany

Extracts from the hemp plant *Cannabis sativa* have been known for centuries to modulate emotional behaviours. The discovery of endogenous “cannabis” receptors (cannabinoid receptors CB1 and CB2) and endogenous “cannabinoids” (called endocannabinoids) and the findings of numerous studies over the last 20 years have fuelled the hope that accumulating knowledge on this novel neuromodulatory system might be transferable from basic research to clinics.

It has emerged, however, during the last years that the endocannabinoid, eCB system consists of several components and is built up in a highly complex manner. Consequently, basic

research has taken a considerable effort in order to understand the “logic” of this neuromodulatory system. This is the prerequisite for the development of rationalized therapeutic strategies targeting the endocannabinoid system.
The following important features of the eCB system have to be considered. eCBs (2-arachidonoylglycerol and anandamide) and CB1 receptors, i.e. the major cannabinoid receptor in the brain, are expressed presynaptically in many different neurotransmitter systems, whereby eCBs reduce neurotransmission: the glutamatergic, GABAergic, cholinergic, noradrenergic, and probably also serotonergic system. Thus, the eCB system represents a “break“-mechanism. Depending on which neurotransmitter system is active in a particular behaviour, it may be favourable to enhance or to diminish neuronal circuit activity. Based on this concept, it has emerged that pure CB1 receptor agonists are very problematic as therapeutic reagents, as they bind to and activate all CB1 receptors, irrespective of the activity pattern of the eCB system in a particular behaviour. Thus, currently, the most promising strategy is to influence biosynthetic and/or degradation pathways of eCBs. In fact, agents inhibiting the anandamide degrading enzyme fatty acid amide hydrolase (FAAH) have been shown to display very good anxiolytic activities.

Discussions
David Finn (Ireland) gave the first discussion presentation. Based on the high co-morbidity between pain and anxiety disorders he posed the question whether there is a role for the endocannabinoid system at the point where pain and anxiety converge. Studies from his laboratory, using a rat fear-conditioned analgesia paradigm, clearly demonstrate that blockade of CB1 receptors systemically or at the level of the dorsolateral periaqueductal grey prevents fear-conditioned analgesia. The role of CB1 is further substantiated by the finding that inhibition of FAAH with URB97 enhances fear-conditioned analgesia via activation of CB1. Evidence was also presented demonstrating that blockade of CB1 receptors in the basolateral amygdala enhances conditioned fear responding and attenuates the suppression of fear responding normally observed in the presence of formalin-evoked nociceptive tone. Studies of the effects of intra-ventral hippocampus administration of URB597 and rimonabant suggested that this region may also play a role in endocannabinoid-mediated fear-conditioned analgesia. It was concluded that FAAH inhibition may be a promising avenue for the development of anxiolytic and analgesic drugs but it was added that more knowledge regarding the regulation of the balance between fear and pain by endocannabinoids is urgently needed.

Next Maria-Paz Viveros (Spain) discussed some additional very important issues in endocannabinoid research. She emphasised that the diverse nature of anxiety disorders makes it important to use a battery of anxiety tests to study the role of the endocannabinoid system and not to rely on one test exclusively. Furthermore, she put forward evidence demonstrating that the exact role of the endocannabinoid system depends on the context of the test, for example the illumination conditions, and the previous experience of the animals, e.g. their exposure to (chronic) stress. Importantly, the effects of CB1 agonists were found to be extremely dose-dependent often resulting in biphasic dose-response curves; although the underlying mechanisms are still unclear it is thought that differential effects of CB1 receptors located on glutamatergic and GABAergic neurons may play a defining role. Finally, evidence was presented showing that both gender and age influence the modulation of anxiety by the endocannabinoid system.

The main theme during the plenary discussion was the question whether it would be feasible to develop anxiolytic drugs targeting the endocannabinoid system given the very opposite effects often generated via CB1 receptors located on glutamatergic versus GABAergic neurons. It was felt that more selectivity would be crucial and that more basic research would be needed to

achieve this. It would be particularly important to better understand the dysregulation of the endocannabinoid system under pathological conditions, such as during chronic stress. It was felt that FAAH inhibition may be a more promising way forward than direct agonism/antagonism of CB1 receptors, although there were concerns that chronic elevation of the endocannabinoid levels could lead to toxic or other side effects. In this respect, it was pointed out that recent results of human studies may indicate that FAAH inhibition could lead to cardiovascular disease due to increased peripheral endocannabinoid signalling. Furthermore, to increase our understanding of the role of endocannabinoids in anxiety it will be important to take the effects of context, experience, gender and age into account when designing experiments in both animals and humans. The participants agreed that there was an immediate need for more studies in humans.

Topic 3 – Posttraumatic stress disorder: neurotransmitters, neuromodulators and hormones – avenues for new drugs?

Main Lecturer: Kerry Ressler, USA

Basic research in the pharmacology of fear and its extinction has direct relevance to psychotherapy of fear-related disorders. Memory enhancing agents in combination with cognitive behavioural therapy hold promise for the treatment of a range of psychiatric disorders. Animal research initially showed that augmentation of extinction learning could be achieved with the NMDA partial agonist, D-cycloserine. This was followed by successful demonstration that D-cycloserine, combined with exposure-based psychotherapy, improved outcomes in certain anxiety disorders. This lecture reviewed recent work exploring a range of possible new strategies in addition to D-cycloserine for the treatment of fear-related disorders, particularly PTSD. These new approaches are based on advancing understanding of the molecular and cellular underpinnings of extinction of fear. Some of these advances include regulation of brain derived neurotrophic factor (BDNF), cannabinoid and cholecystokinin receptors, adrenergic receptor modulators such as yohimbine, and other putative mechanisms for promoting emotional learning in animal models. In summary, advances in the understanding of the neurobiology of fear and extinction of fear learning may lead to exciting and powerful new approaches to treating PTSD and other anxiety disorders.

Discussions

As there is increasing evidence for an important role of noradrenaline and glucocorticoids in fear learning, Gustav Schelling (Germany) first discussed studies on the effects of administration of propranolol and cortisol on the development of PTSD in cardiac surgery patients. The beta blocker propranolol seemed to block traumatic memories from the intensive care unit in female but not in male patients. Interestingly, his group demonstrated that high doses of cortisol, given before and up to 3-4 days after surgery reduce PTSD stress symptoms in cardiac surgery patients. Next, he discussed recently published data showing that endocannabinoids are instrumental in the effects of glucocorticoids on memory consolidation in rats. These effects take place at the level of the basolateral amygdala and involve changes in both GABA and noradrenaline release. Data were presented showing that different forms of stress affect the circulating levels of endocannabinoids also in humans. Importantly, particularly with respect to drug development, data were presented showing that patients with coronary heart disease have increased 2-arachidonoylglycerol levels. Thus, special care should be taken to avoid side effects at the level of the cardiovascular system when targeting the endocannabinoid system for the treatment of psychiatric disorders.

Next, Joseph Zohar (Israel) discussed timing issues related to therapeutical interventions for the treatment of PTSD. He made the point that there exists a so-called ‘window of opportunity’ for

successful treatment. He argued that debriefing and sleep during the hours immediately after the traumatic event may worsen the outcome and enhance the risk of the development of PTSD. He further discussed translating human PTSD into an animal model making use of the variability between individuals in the response to anxiety tests such as the elevated plus maze and acoustic startle after having experienced a traumatic event (exposure to predator odour). This is particularly important as in humans only a small proportion of subjects exposed to a traumatic event will eventually develop PTSD. Using this animal model, he demonstrated that one high dose of corticosterone, but not a low dose, administered one hour after trauma exposure reduces PTSD-like behavioural symptoms. Importantly, he also showed that treatment with benzodiazepines immediately after the exposure may lead to more extreme behavioural responses. Going back to human studies, the design of a recently completed, prospective study on the effects of escitalopram was presented, the findings of which are expected soon.

One of the main themes of the plenary discussion was whether there exists a relationship between the development of PTSD and the experience of pain. There seems to be indeed a higher chance to develop PTSD when there is both psychological and physical trauma. It was noted that most compounds discussed in this session, including SSRIs and glucocorticoids, have analgesic effects. The participants agreed that more studies would be needed to further elucidate this relationship.
The second main discussion topic was whether PTSD should be regarded a memory disorder rather than an emotional disorder. There was agreement that the large body of evidence on fear memory and fear extinction as presented during this TEM certainly favours such hypothesis. Furthermore, it was noted that patients with amnesia are less likely to develop PTSD. There was an interesting suggestion that having extensive posttraumatic events to remember may compete with the actual traumatic memory. The notion that social support is crucial in the period immediately after the traumatic event may underscore this idea and it was proposed that studies in animals, using enriched environments, may provide further insight.

II. Future topics and concluding remarks

At the closure of the meeting there was a final discussion on the topics in the field of anxiety disorders and anxiolytics that were considered important for further discussion and research:

- It was evident, also from the discussions during this TEM, that more cross-talk between pre-clinical and clinical scientists is crucial. How can human behaviours be translated into animal models? And also vice versa: what can we learn from animal models, to design improved and more detailed and focused studies in human volunteers and patients with anxiety disorders? In this respect it was noted that animal studies should preferentially use a battery of tests, not only covering different fear and anxiety dimensions, but also, with respect to the high co-morbidity between anxiety and depression, including aspects of affective behaviour.
- It was also noted that, given the limited access to biological specimens in clinical research, it would be essential to further develop imaging strategies and invest in the identification of biomarkers for the various anxiety disorders.
- Finally, the need for an integrative neurobiological model, bringing together the present knowledge on the role of the different neurotransmitters and neuromodulators in the regulation of fear and anxiety was emphasised.

Last but not least, the organisers, the speakers, the discussants and the participants would like to thank the ECNP for this great opportunity to come together and intensively discuss the new developments in the field. It was an extremely valuable experience.

III. Participants

Lecturers, discussants, coordinator and co-chair:
David Baldwin (UK), David Finn (Ireland), Astrid Linthorst (UK), Beat Lutz (Germany), Sanjay Mathew (USA), Kerry Ressler (USA), Gustav Schelling (Germany), Thomas Steckler (Belgium), Dai Stephens (UK), Maria-Paz Viveros (Spain), Joseph Zohar (Israel).

Further participants:
Catherine Belzung (France), Johan den Boer (The Netherlands), Damiaan Denys (The Netherlands), Julia Fedotova (Russia), Matthew Garner (UK), Andrew Gloster (Germany), Eva Maria Marco López (Spain), Giovanni Marsicano (France), Agnes Nocon (Germany), Stefano Pallanti (Italy), Michelle Roche (Ireland), David Slattery (Germany), Nic van der Wee (The Netherlands).

TEM symposium at the 23rd ECNP Congress:
Neuropsychobiological mechanisms underlying fear memory and extinction: options for new treatment strategies

chairs: Johan A. den Boer, The Netherlands; Astrid C.E. Linthorst, United Kingdom

- Fear memory and extinction: clinical and therapeutic aspects
  t.b.a.
- Neuronal signalling and epigenetic mechanisms at the cognition-emotion interface
  Johannes M.H.M. Reul, United Kingdom
- The role of endocannabinoids in the amygdala in the regulation of emotional memory
  Patrizia Campolongo, Italy
- Glucocorticoids and noradrenaline in severe stress exposure in humans
  Gustav Schelling, Germany
- D-cycloserine and exposure therapy
  Barbara Rothbaum, USA