351P NEUROBIOLOGY OF ANXIETY AND PANIC: THE ANIMAL APPROACH

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The neurobiology of anxiety and panic is an area in which animal studies have made a major contribution. Indeed, our understanding of the brain circuitry involved has come largely from animal models, although these data are now beginning to be backed up by human brain-imaging.

The capacity to overcome or avoid sources of danger is essential to survival: powerful and well-validated animal paradigms have thus been developed which model what might be regarded as 'normal' fear and anxiety. Their origins date back to the instrumental approach-avoidance conflict tests of the 1970s which were followed by more ethologically-based models such as social interaction and elevated plus-maze (Handley, 1995).

Relative insensitivity to benzodiazepines discouraged the use of conditioned emotional response (CER) paradigms for pharmacological studies and it is only recently that any understanding has been achieved of how their neurobiology differs from the conflict tests (Killcross *et al*, 1997). Meanwhile, individual components of behaviour, including freezing and startle responses, provided a productive substrate to delineate the pathways involved in learned and innate responses to aversive stimuli.

These models have revealed an anxiety substrate encompassing virtually all levels of the neuraxis but most notably focussed on pathways extending from the amygdala and hypothalamus to the periaqueductal grey (LeDoux, 1996). These may be entered at different levels according to the stimulus: complex events may require cortical evaluation while certain discrete stimuli can access lower levels directly and learned signals may be treated differently from unlearned ones. Output pathways include those governing inhibition and activation of motor output as well as endocrine and autonomic systems.

With this degree of complexity, it is no surprise that the neurotransmitter regulation of anxiety and panic is itself heterogeneous. While the role of GABA is prominent, many other transmitters have been implicated. The role and importance of these transmitters can appear to vary according to the model and conditions used, emphasising that anxiety is unlikely to be controlled by a single isolated system but rather by a network of subsystems working together to provide the optimum outcome in terms of survival (McCreary *et al.*,1996).

The challenge now is to map human pathology onto the neurobiology revealed by animal modelling and thus to isolate procedures and paradigms most suitable for use in deriving therapeutic strategies.

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352P NEUROBIOLOGY OF ANXIETY AND PANIC: HUMAN STUDIES

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Symptoms of anxiety are common to a number primary anxiety disorders which are distinguished more by the triggers for the symptoms than the symptoms themselves. Generalised anxiety disorder (GAD) involves sustained autonomic symptoms, muscular tension and subjective apprehension and worry. In contrast, panic disorder (PD) involves brief but intense attacks which may be spontaneous or linked to triggers such as crowds.

Twin studies suggest that GAD and PD involve distinct genetic mechanisms and that GAD is a variant of depression (Kendler *et al*, 1996). Indeed, some allelic variants of the 5-HT transporter may be associated with risk of depression and with neuroticism, a personality dimension related to GAD (Lesch *et al*, 1996). The associations with 5-HT alleles are compatible with many neuroendocrine studies showing impaired presynaptic 5-HT function in depression, but whether this occurs in GAD is not clear

GAD and PD respond to antidepressants which increase 5-HT function, and this suggests both are modulated by 5-HT. However, GAD and PD respond differently to other 5-HT drugs. GAD and depression respond to the 5-HT_{1A} agonist buspirone, and 5-HT_2 antagonists may be anxiolytic and antidepressant. In contrast, these treatments do not improve and may worsen PD. This suggests that PD and GAD involve different afferent pathways to anxiety effectors, which former are differently modulated by 5-HT.

Panic attacks can be provoked in volunteers by cholecystokinin-related peptides by lactate and by CO_2. PD patients are more sensitive to these agents but this may also be true of other anxiety disorders such as social phobia and obses-sional compulsive disorder (OCD). Drug trials have failed to demonstrate efficacy of CCK antagonists but this may be due to poor bioavailability

Social phobia is characterised by blushing and other visible anxiety symptoms when under social scrutiny. While response to MAOIs has been emphasised, there is no clear evidence for lack of efficacy of other antidepressants, and MAOIs are effective in other forms of anxiety.

OCD clearly is specifically responsive to serotonin-enhancing antidepressants and not to antidepressants with selective actions on noradrenergic mechanisms. However. while tryptophan depletion causes temporary reversal of antidepressant effects, anti-obsessional effects are not reversed. This suggests that antidepressants may have more than one therapeutic mode of action in anxiety disorders and depression.

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353P NOVEL NEUROPHARMACOLOGICAL APPROACHES TO DRUG DEVELOPMENT

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Anxiety disorders represent one of the most common forms of mental illness with a lifetime prevalence of 5% for generalized anxiety disorder (GAD), 3% for panic disorder (PD), 3-13% for social phobia and 10-11% for specific phobias.

Current theories propose that anxiety may be differentiated into conditioned fear responses controlled by higher centres of the brain (dysfunction of which may result in the excessive worry associated with GAD) and unconditioned fear responses mediated by lower brain regions such as the PAG (dysfunction of which may result in PD). The involvement of multiple brain regions in anxiety responses implies a role for multiple neurotransmitter systems and this has been born out by the clinical effectiveness of drugs that interact with different systems.

For many years the benzodiazepines, such as diazepam, have been the principal treatment for anxiety disorders. These act by potentiating the actions of the inhibitory neurotransmitter, GABA, and are effective in the treatment of GAD and phobias but are generally thought to be less effective in PD. Unfortunately, benzodiazepines are associated with sedation, muscle relaxation and alcohol interaction, while long term use can engender dependence.

More recently, 5-HT reuptake inhibitors have become the treatment of choice for PD. However, these are not effective in all patients, have delayed onset of action and are also associated with side effects, particularly nausea, insomnia, tremor and sexual dysfunction. Thus, novel therapies with immediate onset of action, high efficacy and with few side effects are still required.

Of the most recent approaches. 5-HT_3 receptor antagonists are effective in some animal models, but there are few reports of clinical efficacy. 5-HT_{1A} receptor agonists, such as buspirone, are effective treatments of GAD but not of PD, and are poorly tolerated. Considerable effort has been directed at the CCK_B receptor antagonists, as CCK infusion causes panic attacks in panic patients. However, these have not had any positive effect in clinical trials to date while other main areas of research, such as CRF antagonists, are as yet clinically untested.

Another approach has centered on the development of selective 5-HT_{2C} receptor antagonists. These have anxiolytic-like effects in a wide number of animal tests with a low propensity for side effects, suggesting that they may indeed be useful in the treatment of anxiety.

354P TREATMENT OF ANXIETY AND PANIC

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During the last decades there has been an ongoing search for the ideal anxiolytic drug. Benzodiazepines are the most widely used anxiolytics, but clinicians increasingly prescribe benzodiazepines somewhat hesitantly because of their abuse potential. Moreover, benzodiazepines have been used with limited success in anxiety disorders other than generalized anxiety disorder.

Although originally developed as antidepressants, some tricyclic antidepressants (TCAs) and most serotonin reuptake inhibitors (SSRIs) have rapidly gained popularity in the treatment of anxiety disorders. A turning point in the growing awareness of their anxiolytic efficacy was the delineation of panic disorder as a separate diagnostic entity in the DSM-III. Imipramine was the first antidepressant shown to be effective in what would now be classified as PD. Its efficacy has since become well established in many placebo-controlled studies with 70 to 80% of patients experiencing marked to moderate improvement.

Among the other TCAs, clomipramine is the most extensively studied for its potential utility in panic disorder, and it is widely used for this purpose. Studies comparing clomipramine with imipramine indicate that they are broadly equivalent in terms of reducing panic attacks and improving avoidance behaviour and anticipatory anxiety, although it has been suggested that clomipramine has a faster onset of action. Evidence for the efficacy of other TCAs is sparse, but we have shown that maprotiline is probably not effective (Westenberg & den Boer, 1993). The maior difference between maprotiline and drugs like clomipramine is that the former does not inhibit serotonin uptake. This notion led us to conclude that the efficacy of antidepressants in panic disorder probably entails alterations of the serotonergic system in the brain. More recent studies with SSRIs havc generated sufficient data to confirm this hypothesis. By and large, SSRIs have demonstrated efficacy in patients with panic disorder) most of whom also had agoraphobia (Westenberg, 1996).

A major problem in evaluating panicolytic or anxiolytic efficacy of antideprcssants in anxiety disorders is the presence of depression as a comorbid condition. Epidemiological studies have shown that panic disorder and unipolar depression occur more commonly together than could be explained by chance. The failure of some antidepressants to improve symptoms in patients with panic disorder and the finding that antidepressants are also efficacious in patients without symptoms of depression, indicates that antidepressants have specific anxiolytic or panicolytic properties. The anxiolytic efficacy seems to extend to other anxiety disorders.

There is now circumstantial evidence that SSRIs are also efficacious in social phobia. Controlled studies indicate that antidepressants may alleviate social anxiety and social avoidance in generalized social phobias. The utility of antidepressants in generalized anxiety disorders has not been adequately studied. Some TCAs have proven to be efficacious in this condition, but with a delayed onset of action. Studies investigating the anxiolytic potential of SSRIs in generalized anxiety disorders are very limited.

Given all of the above, it appears that antidepressants, and particularly those that potently inhibit the serotonin uptake, may have 'true' anxiolytic activity irrespective of the nosological background. A major disadvantage is the absence of an immediate calming effect typical for benzodiazepines. To achieve rapid relief of symptoms, a combined therapeutic strategy may prove beneficial fior some patients.

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355P TREATMENT OF ANXIETY AND PANIC: FUTURE DIRECTIONS

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There is now ample evidence, both preclinically and clinically, that the $GABA_A$-benzodiazepine receptor complex and the serotonergic (5-HT) system, are crucial in the modulation of anxiety and fear.

Agonists of the 5-HT_{1A} receptor (e.g. 8-OH-DPAT, flesinoxan, buspirone) have anxiolytic activity in most animal models of anxiety (e.g. conflict procedures, elevated plus maze, light-dark box, stress-induced hyperthermia, distress vocalizations). Recently, 5-HT_{1A} receptor antagonists have become available (WAY 100,635, S-UH 301, DU 125530) which were initially qualified as "silent". We tested several 5 HT_{1A} receptor antagonists in various animal paradigms of anxiety, including ultrasonic distress calls in adult rats and the fear-potentiated startle test in rats.

Surprisingly, WAY 100,635 had an anxiogenic effect at lower doses, which faded away at higher doses, in the ultrasonic distress test (Groenink *et al*, 1996). Conversely, three 5-HT_{1A} receptor antagonists (WAY 100,635; (+)pindolol and DU125630) had anxiolytic activity in the fear-potentiated startle test (Joordens *et al* 1997). In a number of other anxiety paradigms (stress-induced hyperthermia, ultrasonic vocalizations in pups, Geller-Seifter conflict procedure), 5-HT_{1A} receptor antagonists were devoid of intrinsic activity. It can be hypothesized that, depending on the tone of the serotonergic system, 5-HT_{1A} receptor antagonists may exert anxiety-modulating effects.

It seems worthwhile to study such compounds in a variety of human anxiety disorders, because the intrinsic activity of the 5-HT systems may vary considerable under different conditions.

Genetic manipulations of the serotonergic system may be of further help to unravel the role of (parts of) this system in anxiety and fear processes.

Recently, the 5-HT_{1B} receptor knock-out (KO) mouse has been introduced (Sau-dou *et al* 1994). The 5-HT_{1B} receptor contributes an important feedback system on the release of 5-HT from serotonergic terminals (autoreceptor) and also as an heteroreceptor on other neurons (eg ACh, NA).

5 HT_{1B} KO (compared to u/wildtype (WT) mice) showed a deviant circadian rhythm (telemetrically measured) of heart rate, body temperature and activity. In the stress-induced hyperthermia paradigm, KO's had higher basal body temperatures than WTs, but were equally sensitive to the temperature-lowering effects of the 5-HT_{1A} receptor agonist flesinoxan. KO's were very sensitive to mild stressors and can be characterized as more "impulsive" than WT's. Apparently, the permanent loss of the "inhibitory" 5-HT receptor has led to permanent and rather drastic changes in behaviour.

Whether the 5-HT_{1B} KO mouse can be used as an animal model of impulsiveness (OCD?) is a matter of further investigations.

It can be concluded however, that 5-HT_{1A} and 5-HT_{1B} receptors are of high interest in the area of anxiety, aggression, stress and depression.

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356P MOLECULAR MODELLING OF P-450 SUBSTRATE INTERACTIONS

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Development of three-dimensional models of human cytochromes P450 involved in drug metabolism, using sequence homology with a unique bacterial P450 template, is discussed.

With the inclusion of experimental information from site-directed mutagenesis and other data, it is found that the homology models generated are consistent with results on specific P450 substrate metabolism.

Key determinants of P450 substrate specificity appear to be associated with the spatial disposition of certain amino acid residues lining the respective P450 binding sites which are complementary to similar groupings on the substrate molecules. These key interactions orientate P450 substrates for metabolism at the experimentally observed positions.