Anxiolytics: mechanisms

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No conflict of interest
Anxiety versus fear

- **ANXIETY**
  - anxious apprehension and worry that is a more general reaction that is out of proportion to threats in environment
  - future oriented
  - can be adaptive if not excessive

- **FEAR**
  - Experienced when a person is faced with real and immediate danger.
  - Present-oriented
  - Can be adaptive
Fear and anxiety

The prefrontal cortex regulates the expression of fear based on previously learned information. Recently, this brain area has emerged as being crucial in the initial formation of fear memories, providing new avenues to study the neurobiology underlying aberrant learning in anxiety disorders.
Long duration fear stimulus

Basolateral Amygdala

Cortex Hypervigilance?

Glu ?

Med. Central Amygdala

Lat. Central Amygdala

CRIF

Glu

Lateral BNST

lateral bed nucleus of the stria terminalis

Fear

Anxiety
Pathophysiology of anxious disorders

Abnormal regulation of neurobiological substrates:

- 5-HT, GABA, Glutamate
- Autonomic nervous system
- Hypothalamo-hypophysis axis
- Neuropeptides: CCK, P substance, galanin.....
# Neuromediators in the brain (μmol/g)

## Amino Acides (70-90 %)
- **Glutamate**: $14 \times 10^6$
- **Aspartate**: $4 \times 10^6$
- **GABA**: $2,5 \times 10^4$
- **Glycine**: $2 \times 10^6$

## Amines (5-20 %)
- **Acetylcholine**: $25 \times 10^3$
- **Dopamine**: $6,5 \times 10^3$
- **Serotonin**: $2,5 \times 10^3$
- **Histamine**: $1 \times 10^3$

## Neuropeptides (< 5 – 10 %)
- **CCK**: 470
- **Met-enkephalin**: 350
- **Somatostatin**: 30
- **Substance P**: 100
- **Neurotensin**: 12
- **VIP**: 40
### Monoaminergic functions

<table>
<thead>
<tr>
<th>NA</th>
<th>5-HT</th>
<th>DA</th>
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<tbody>
<tr>
<td>Vigilance</td>
<td>Impulsivity</td>
<td>Vigilance</td>
</tr>
<tr>
<td>Motivation</td>
<td>Appetite</td>
<td>Hyperactivity</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>Dependence</td>
</tr>
<tr>
<td></td>
<td>Agressivity</td>
<td></td>
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</table>
SITES OF ACTION OF NEUROTRANSMITTERS
Anxiolytics

• Benzodiazepines and GABA active drugs

• Buspirone: 5HT1a partial agonist

• SSRIs

• Antipsychotics?
Serotonin in anxiety

• Classical hypothesis of anxiety:
  • ↓ serotonin pathways \implies anxiolytic effect
  • ↑ serotonin pathways \implies anxiogenic effect

• Dual hypothesis of anxiety:
  • Role of amygdala and peri-aqueductal gray
• Lesions to the amygdala disrupt the conditioned responses but do not affect the learning of relevant declarative facts.

• Hippocampal lesions disrupt the learning of relevant facts but do not affect the acquisition of conditioned responses.
Serotonergic model of anxiety (Graeff et al 1996)

Frontal Cortex

Ascending pathway facilitates conditioned fear

Dorsal Raphe Nucleus – periaqueductal pathway inhibits inborn inconditioned fear

Dorsal Raphe nucleus

Amygdala

Periaqueductal grey
Benzodiazepines

• BZDs: Anxiety-Reducing Activity by Reduction of Serotonin Turnover in the Brain (Wise CD, Berger BD, Stein L Science. 1972)

• Anxiolytic effects of BZDs are neutralized by intravenous 5-HT

• BZDs: Allosteric modulators of GABA$_A$ receptors
SPECTRUM OF BENZODIAZEPINE RECEPTOR LIGANDS

Intrinsic efficacy

Agonists
Partial Agonists
Antagonists
Partial Inverse Agonists
Inverse Agonists

midazolam  bretazenil  flumazenil  Ro 15-4513  Ro 19-4603
(Ro 16-6028)  (Ro 15-1788)

Benzodiazepine receptor
GABA_A receptor affinity
Chloride channel gating
GABA

✓ G aminobutyric Acid (GABA): one of the most abundant neurotransmitters in the CNS

✓ All the brain structures contain GABAergic neurons

✓ Discovered 50 years ago that GABA is an inhibitor neurotransmitter in the CNS

  ➢ 30% of synapses in vertebrates
GABAergic system (1)

✓ GABA: gamma-aminobutyric acid
  ➢ inhibitor activity
  ➢ 1st amino acid which the neurotransmission role has been recognised
  ➢ [GABA]: more 200-1000 times than Ach. or 5-HT

✓ 3 types of receptors: $\text{GABA}_A$ (ionotropic: coupled to ion channel), $\text{GABA}_B$ (metabotropic: coupled to G protein) and $\text{GABA}_C$
GABAergic system (2)

- **GABA**$_A$ receptor is sensitive to muscimol (agonist), bicuculline and picrotoxin (antagonists)
  - GABA binding leads to opening of Cl$^-$ channel followed by hyperpolarisation of the target cell

- **GABA**$_B$ receptor is sensitive to baclofen (agonist) and CGP 56119 (antagonist)
  - receptor coupled to G protein G0 or Gi
  - G0 protein can be coupled with Ca$^{2+}$ ou K$^+$ channel
GABA_A receptor (1)

- 5 trans-membrane glycoprotein subunits arranged round central chloride channel - ‘ligand-gated ion channel’

- activation $\rightarrow$ chloride influx $\rightarrow$ hyperpolaristion $\rightarrow$ neuronal inhibition

- may have multiple allosteric modulating sites as part of the receptor complex (e.g. benzodiazepines, barbiturates, alcohol)
GABA<sub>A</sub> receptor (2)

- Gaba A site
- Chloride channel
- Picrotoxin site
- Alcohol site
- Barbituric site
- Benzodiazepines site
GABA\textsubscript{A} sub-units

- 19 different sub-units, classified into 6 major classes

- Composition determines pharmacological characteristics

- Function depends on subunits of pentameric complex

- GABA\textsubscript{A} \(\alpha\)1 (60\% of all GABA\textsubscript{A} receptors):
  - sedative, amnestic, anticonvulsant

- GABA\textsubscript{A} \(\alpha\)2 (15\% of all GABA\textsubscript{A} receptors):
  - anxiolytic, muscle relaxant

- GABA\textsubscript{A} \(\alpha\)3 (15\% of all GABA\textsubscript{A} receptors):
  - unknown

However, $\text{GABA}_A$ receptors are ubiquitously expressed in the CNS therefore – side effects
Differential modulation of $\text{GABA}_A$-receptor subtypes

- classic benzodiazepines (e.g. diazepam, temazepam)
  - non-specific activation of all $\alpha$ subtypes

- ‘non-benzodiazepine’ BZD-receptor agonists (e.g. zolpidem, zaleplon)
  - high affinity for $\alpha_1$ subtype

- GABA reuptake inhibitors (tiagabine)
  - enhanced activity at all $\alpha$ subtypes
GABA\textsubscript{A} receptor isoforms

Most current therapeutics interact with many of the ~20 GABA\textsubscript{A} receptor subtypes causing adverse effects.
<table>
<thead>
<tr>
<th>Pharmacological effect</th>
<th>Receptor involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiolysis</td>
<td>$\alpha_2$ containing</td>
</tr>
<tr>
<td>Sedation</td>
<td>$\alpha_1$ containing and those not containing $\alpha_1$</td>
</tr>
<tr>
<td>Anterograde amnesia</td>
<td>$\alpha_1$ containing</td>
</tr>
</tbody>
</table>
## Enhancement of GABA

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased synthesis</td>
<td>Topiramate, valproate</td>
</tr>
<tr>
<td>Inhibit breakdown</td>
<td>Valproate, vigabatrin</td>
</tr>
<tr>
<td>Inhibit reuptake</td>
<td>Tiagabine</td>
</tr>
<tr>
<td>Allosteric GABA&lt;sub&gt;A&lt;/sub&gt; modulation</td>
<td>BZDs, barbiturates, neurosteroids, topiramate</td>
</tr>
<tr>
<td>Direct agonism</td>
<td>Alcohol, high-dose barbiturates, chloral hydrate, abecarnil, pagocline</td>
</tr>
<tr>
<td>GABA analogues</td>
<td>Gabapentin, pregabalin</td>
</tr>
</tbody>
</table>
Mechanism of action of tiagabine
Non-GABA-ergic targets for anxiolytic drugs

- 5-HT₁ and 5-HT₂ receptor antagonists
- melatonin receptor agonists
- antagonists at the substance P (NK-1) receptor
- metabotropic glutamate receptor antagonist
- cholecystokinin antagonists
- neuropeptide Y agonists
- adenosine A₁ and A₂A receptor agonists
5-HT targets for novel anxiolytic drugs

<table>
<thead>
<tr>
<th>Approach</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT_{1A} agonist</td>
<td>Buspirone patch to avoid first-pass hepatic metabolism.</td>
</tr>
<tr>
<td>SSRI plus 5-HT_{2} antagonism</td>
<td>Nefazodone. Relief of anxiety symptoms in major depression and efficacy in panic disorder.</td>
</tr>
<tr>
<td>5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists</td>
<td>May increase 5-HT availability in synaptic cleft and advance onset of action of SSRIs.</td>
</tr>
<tr>
<td>5-HT_{2C} antagonist</td>
<td>Deramiclcline. Proven efficacy in Phase II study not confirmed in subsequent Phase III studies.</td>
</tr>
<tr>
<td>5-HT_{3} antagonist</td>
<td>Claims of efficacy in GAD and panic disorder not supported by subsequent studies.</td>
</tr>
<tr>
<td>5-HT_{2C} antagonist and melatonin agonist</td>
<td>Agomelatine. Proven efficacy in Phase II and III studies in depression. Relief of anxiety symptoms.</td>
</tr>
<tr>
<td>SSRI plus norepinephrine reuptake inhibition</td>
<td>Venlafaxine, milnacipran, duloxetine. Venlafaxine has proven efficacy in GAD, social phobia, panic and PTSD</td>
</tr>
</tbody>
</table>
AGONISM AT THE 5-HT1A RECEPTOR

A. Activation of presynaptic 5-HT1A receptor may reduce 5-HT release

B. Activation of postsynaptic 5-HT1A may explain paradoxical anxiety after acute administration
Limiting role of $5$-$\text{HT}_{1A}$ receptors
Action of SSRIs in GAD

Anxiety level

Treatment period

withdrawal

Week

SSRI

BZD
Antipsychotics as anxiolytics

Atypical antipsychotics such as quetiapine, aripiprazole, olanzapine and risperidone have been shown to be helpful in addressing a range of anxiety symptoms in individuals with schizophrenia and schizoaffective disorders, and have since been used in the treatment of anxiety.
5HT2c and anxiety

As a therapeutic purpose several 5HT2c antagonists were developed for the treatment of several nervous system disorders including anxiety.
Potential impact of melatonin receptor agonists

- **Agomelatine**: melatonin agonist and 5-HT$_{2C}$ antagonist

- superior to placebo in at least 2 placebo-controlled trials $^{1,2}$

- side effect profile similar to placebo at 5-25 mg/day

- no discontinuation symptoms$^3$

$^1$Loo et al., 2002; $^2$Loo et al., 2003; $^3$Montgomery et al., 2004
<table>
<thead>
<tr>
<th>Receptor type</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionotropic (iGlu)</td>
<td>Ligand-gated ion channels. Mediate synaptic excitability and plasticity</td>
</tr>
<tr>
<td>Metabotropic G-protein coupled receptors (mGlu$_{1-8}$)</td>
<td>Regulate glutamate release and modify postsynaptic excitability</td>
</tr>
<tr>
<td>Plasma membrane glutamate transporters (EAAT$_{1-5}$)</td>
<td>Clear synaptic space of released glutamate and other excitatory amino acids</td>
</tr>
<tr>
<td>Vesicular glutamate transporters (vGluT$<em>{1}$ and vGluT$</em>{2}$)</td>
<td>Package glutamate for exocytotic release</td>
</tr>
</tbody>
</table>

Adenosine receptors - a role in anxiety?

• caffeine is non-selective adenosine receptor ($A_1$ and $A_{2A}$) antagonist

• induces wakefulness in wild and $A_1$ receptor knockout mice, with no induction in $A_{2A}$ knockout mice

• wakefulness therefore arises through effects on $A_{2A}$ receptor$^1$

• $A_{2A}$ agonists have potential anxiolytic properties

• possible polymorphism of $A_{2A}$ receptor in panic disorder$^2$

$^1$Huang et al (2005) Nature Neurosci 8: 858-859

SITES OF ACTION OF NEUROPEPTIDES

From Millan, 2003
CHOLECYSTOKININ

• Cholecystokinin is a neuropeptide discovered in the gastrointestinal tract
• Probably the most abundant neuropeptide in the brain
• Two subtypes of receptors are existing: CCK_1 receptors are widely distributed in the periphery and they are established in the distinct brain nuclei, whereas CCK_2 receptors are present in the brain and stomach
Cholecystokinin (CCK) is involved in the regulation of various physiological functions, including pain, feeding, and emotional behaviour. CCK is implicated in the regulation of anxiety. The administration of CCK-4 induced panic attacks in humans and anxiety-like state in animals. Anxiogenic-like action of CCK is mediated via CCK$_2$ receptors. Cholecystokinin is also a co-mediation of GABA in the neurons of hippocampus and cerebral cortex.
Cholecystokinin antagonists

• CCK-4 can provoke anxiety in healthy volunteers

• effects of CCK-4 are attenuated by anti-panic medications

• CCK-4 antagonists block anxiogenic effects of CCK

• no proven efficacy in anxiety disorders
Corticotrophin-releasing factor (CRF) is an neuropeptide that plays a prominent role in the endocrine, autonomic, behavioural and immune responses to stress.
CRF injected into brain of rats produced many of the signs and symptoms seen in patients with anxiety disorders. (Arborelius et al., 1999)
CRF$_1$ KO mice showed anxiolytic behaviour

CRF over expressing mice show anxiogenic-like response
CRF1 antagonists
CP-154,526
Promote anxiolytic responses in:
The elevated plus maze
The mouse defense battery
The fear-patiented startle test
The light dark test
But not in conflict tests
CRF Human studies

Mainly in depression but disappointing
SUBSTANCE P AND NEUROKININS

SP – NK-A – NK-B bind to 6-protein Coupled receptors (NK1-NK2-NK3)

NK1 and NK-3 (septum, striatum, Amygdala, periaqueductal grey matter).

NK2 Lower levels in CNS
Substance P

- 11 amino acid peptide: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂
- Belongs to tachykinine family with NKA and NKB
- Localisation:
  - Septum
  - Hippocampus
  - Striatum
  - Amygdala
  - Substancia nigra
  - PAG
  - NTS
- Physiologic roles:
  - Nociception
  - Inflammation
  - Anxiety, depression?
### NK and MT agonists

<table>
<thead>
<tr>
<th></th>
<th>NK&lt;sub&gt;1&lt;/sub&gt;</th>
<th>MT&lt;sub&gt;1/2&lt;/sub&gt;</th>
<th>Agonists MT&lt;sub&gt;1/2&lt;/sub&gt; + DZP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vogel</td>
<td>0</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>EPM</td>
<td>0</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Hypophagia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T maze</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>
GALANIN

- Administered directly into the central nucleus of the amygdala blocked the anxiogenic effect of yohimbine.
- GAL-R1 deficient mice show increased anxiety-like behaviour.
- Behavioural response to stress may depend on the balance between NA, NPY and galanin.
ATRIAL NATRIURETIC PEPTIDE AND PANIC DISORDER

• Pretreatment of 150 microg of atrial natriuretic peptide protected against CCK-4 induced panic in PD patients. Strohle et al Am J Psychiatry 2001, 158, 1514-1516

• As well in healthy volunteers Wiedmann et al Arch Gen Psychiatry 2001, 58, 371-377
Vasopressin Antagonist

• The Vasopressin V1b Receptor Antagonist SSR149415 in the Treatment of Major Depressive and Generalized Anxiety Disorders: Results From 4 Randomized, Double-Blind, Placebo-Controlled Studies

• Guy Griebel, PhD; Sandra Beeské, MS; and Stephen M. Stahl, MD
Neuropeptides in anxiety

• Less important than GABA and Serotonin

• They play like dimmer, not like light producers
## Conclusion of anxiolytics

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<th>CLASSES OF ANXIOLYTICS</th>
<th>USES</th>
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<tr>
<td>Benzodiazepines</td>
<td>Generalized anxiety disorders, OCD, phobia, panic attack</td>
</tr>
<tr>
<td>SSRIs</td>
<td>Generalized anxiety disorders, OCD, phobia, panic attack</td>
</tr>
<tr>
<td>Tricyclic antidepressants (doxepin, imipramine)</td>
<td>Anxiety with depression, panic attacks</td>
</tr>
<tr>
<td>5HT1A agonists (Buspirone)</td>
<td>Mild anxiety, Not effective in panic attack</td>
</tr>
<tr>
<td>Beta blockers (propranolol, atenolol)</td>
<td>Social anxiety</td>
</tr>
<tr>
<td>MAO inhibitors Phendelzine</td>
<td>Panic attack, phobia</td>
</tr>
</tbody>
</table>
CONCLUSION

• The neurocircuitry of fear and anxiety is complex
• Neuropeptides seems to be regulators of the monoamine transmitters

• There is no yet drug acting on neuropeptides receptors active as anxiolytic
Most highly cited journal for drug discovery
Peer reviewed for authoritative coverage

20th Anniversary of Combinatorial

- Dynamic combinatorial chemistry
- Combinatorial chemistry in anti-infective drug discovery
- Statistical design in combinatorial chemistry
- Predictive ADME simulation in drug discovery

CUVEE DU PHARMACOLOGUE