Looking Beyond the Monoamine Hypothesis

a report by
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The monoamine hypothesis has dominated research into the pathophysiology and pharmacotherapy of depression for a long time. This has led to the development of antidepressants that are now more selective than the early tri- and tetracyclics from which they have evolved. Alternative hypotheses such as those involving adult neurogenesis or components of the hypothalamic–pituitary–adrenal (HPA) axis are either too premature or have not led to drugs with improved antidepressant activity. Recent new approaches include DNA techniques (identifying genes and gene expression)[1,2] and proteomics (a complete inventory of all proteins).[3,4] To date, they have not contributed to the development of new drugs. Although many exciting developments are occurring, it does not appear as easy to develop the next generation of antidepressant drugs that do not influence monoamines. In the meantime there may be no choice other than to make the best of the existing hypotheses. This is not as hopeless as it may seem, because there is still considerable potential in the concept of monoamine reuptake inhibition.

Monoamines, Neuroimaging and Sub-components of the Depressive Syndrome

The monoamine hypothesis of depression[5] does not only propose the crucial involvement of monoamines in the therapeutic effects of antidepressant drugs but also suggests that depression is directly related to decreased monoaminergic transmission. In view of recent developments in molecular biology, it is relevant to consider what the actual position of this hypothesis is and whether recent findings (e.g. based on neuroimaging techniques) still support its validity.

There are new data that fit well into the monoamine hypothesis. Many of them originate from positron emission tomography (PET) studies. By using selective radioligands, evidence was found for reduced pre- and post-synaptic 5-hydroxy-tryptamine $(5\text{-HT})_{1A}$ receptor binding in depression. Drevets et al.[6] demonstrated that the mean 5-HT_{1A}-receptor-binding potential (BP) was reduced in the mesiotemporal cortex and raphe area in unmedicated depressives relative to controls using PET and (^{11}C) WAY-100635. A similar reduction was evident in the parietal cortex, striate cortex and left orbital cortex/ventrolateral pre-frontal cortex. These data are consistent with those of Sargent et al.,[7] who found decreased 5-HT_{1A}-receptor-binding potential (BP) in unmedicated depressed patients relative to healthy controls in the raphe, mesiotemporal cortex, insula, anterior cingulate, temporal polar cortex, ventrolateral pre-frontal cortex and orbital cortex. However, a subgroup of the subjects was scanned both pre- and post-paroxetine treatment and the 5-HT_{1A} receptor BP did not significantly change in any area.

Most brain imaging studies conducted in patients with major depression episodes (MDE) have been able to identify abnormalities associated with MDE.[8,9] There is a large inter-individual variability in severity and psychopathological features associated with MDE. This might be related to the habit of treating major depression as a unitary construct, while recent evidence suggests that depression consists of several sub-components.

A recent PET study with ^{11}C-labelled 3-amino-4-(2-dimethylaminomethylphenylsulfanyl) benzonitrile ((^{11}C)DASB), a selective radioligand for the 5-HT transporter (5-HTT), in patients suffering from MDE investigated the contribution of another factor associated with depressed moods – namely, the presence of dysfunctional attitudes and the relationship thereof with the 5-HTT binding potential.[10] Dysfunctional attitudes are negatively biased assumptions and judgements about the world and oneself and constitute a negative cognitive interpretative bias of the future. Most studies have investigated the relationship with depression as a syndrome and have ignored the presence of other variables such as dysfunctional attitudes. Interestingly, no differences in 5-HTT BP were found among the entire sample of depressed patients compared with healthy controls. Depressed patients with high regional 5-HTT BP (up to 21%) had higher levels of dysfunctional attitudes. It has been suggested that an increased density of the 5-HTT may lead to increased 5-HT

clearance from the synapse, leading to reduced availability of synaptic 5-HT.

Milak et al.[11] have investigated the association between different psychopathological clusters of the Hamilton Depression Rating Scale (HDRS) and resting glucose metabolism using 18 fluoride-fluorodeoxyglucose ((^{18}F)-FDG) PET. They found distinct correlations between three HDRS factors and regional glucose metabolism. The first factor, psychic depression, showed a positive correlation with metabolism in the basal ganglia, thalamus and cingulate cortex. The second factor, sleep disturbance, showed a positive correlation with metabolism in limbic structures and basal ganglia, and the third factor, loss of motivation, was negatively correlated with parietal and superior frontal cortical areas. Interestingly, this study shows that positive correlations with aspects of depression severity are subcortical ventral, ventral pre-frontal and limbic structures, whereas negative correlations are found in dorsal cortical areas.[11]

According to these neuroimaging studies, serotonin is likely to play a role in the neurobiology of depression in at least a subgroup of patients, but is not necessarily confined to the syndrome of depression. A more fruitful approach would be to search correlates between processes in the brain and subcomponents of MDE, such as motivation, anhedonia, depressed mood, dysfunctional attitudes and sleep disturbances, instead of trying to find neuronal correlates for depression as a syndrome.

Tryptophan Depletion

Studies using tryptophan depletion support the role of serotonin in the modulation of mood, as witnessed by the ability of tryptophan depletion to lower mood.[12] Smith et al.[13] studied the effect of relapse following tryptophan depletion on the cognitive function of depressed patients. They reported an attenuation of the task (verbal fluency) and related activation in the anterior cingulate during relapse, which was correlated to an increase in depressive symptoms. In addition, tryptophan depletion produced a transient exacerbation of depressive symptoms in obsessive-compulsive disorder (OCD) patients responding to selective serotonin reuptake inhibitors (SSRIs).[14] Moreover, inhibition of 5-HT synthesis by p-chloropheny-lalanine or L-tryptophan depletion[15–18] caused a relapse of symptoms in depressed patients who were successfully treated with SSRIs.[19,20] Studies that interfere with the synthesis of serotonin clearly demonstrate that this neurotransmitter plays a crucial role in the therapeutic effect of SSRIs.

Some studies therefore support the monoamine theory of depression, although evidence is also accumulating against a direct relationship between depression and a monoamine deficiency.[21,22] For example, current evidence concerning serotonin does not imply depression but rather aggressiveness, failing impulse control and violent suicide as directly related to impaired brain serotonergic function.[23] Moreover, most antidepressant drugs do not limit therapeutic action to depression, but are also successfully applied in anxiety disorders.[24–29] Differences in gene polymorphism and subsequent differential protein expression of the serotonin transporter do not appear to be related to familiar depression.[30] However, a recent study indicates that the number of life events in combination with a 5-HTT polymorphism could be an important factor in precipitating symptoms of depression.[31] Although the monoamine hypothesis has been challenged by several new data, it can safely be concluded that there is sufficient evidence to support its use as conceptual framework, in particular for pharmacotherapy.[32]

New Developments in Augmentation Strategies

5-HT_{1A} receptor partial agonists have weak antidepressant properties.[33–36] Pre-clinical studies have shown that 5-HT_{1A} receptor agonists induce the rapid desensitisation of 5-HT_{1A} receptors.[37] In theory, co-administration with a 5-HT_{1A} receptor partial agonist may improve the efficacy of an SSRI and reduce its lag time, depending on the size and rate of desensitisation induced by the 5-HT_{1A} receptor partial agonist. Clinical evaluation of this concept has shown beneficial effects, although the evidence is not conclusive.[38–41]

Augmentation with 5-HT_{1A} and 5-HT_{1B} Receptor Antagonists

Microdialysis studies in rats have shown that the increase in extracellular 5-HT elicited by a single dose of an SRI is augmented by co-administration of a 5-HT_{1A} receptor antagonist.[42–47] In addition to the somatodendritic 5-HT_{1A} autoreceptor-mediated feed-back, 5-HT release is also controlled by terminal 5-HT_{1B} receptors. A microdialysis study in ventral hippocampus has compared augmentation by a 5-HT1A with a 5-HT_{1B} receptor antagonist.[47] Augmentation by the 5-HT_{1B} receptor antagonist occurred irrespective of the dose of SSRI. However, in cases of the 5-HT_{1A} receptor antagonist, augmentation was only seen at the highest doses of SSRI.

Clinical Studies with Pindolol

A preliminary study with previously untreated depressed patients suggested improvements in both latency and efficacy by combining treatment with paroxetine and pindolol.[48] Since then, many open-

label and controlled studies with pindolol have followed, albeit with variable success.[49] It soon became evident that the observed clinical effects of pindolol co-administration could not readily be explained by complete antagonism of somato-dendritic 5-HT_{1A} receptors. Based on pre-clinical studies, evidence has been obtained that pindolol may exert its activity through the β-adrenergic receptor and not by means of an interaction with the 5-HT_{1A} receptor.[50]

Augmentation by 5-HT2C Receptor Antagonists – Pre-clinical Studies

Recently, evidence was presented for a novel augmentation strategy based on 5-HT_{2C} receptor antagonism.[51] Augmentation of extracellular 5-HT was observed in rat hippocampus and cortex with citalopram, sertraline and fluoxetine. The effect was at least of a similar magnitude to that seen with 5-HT_{1A} and 5-HT_{1B} receptor antagonists.47 Genetic elimination of these receptors in mice (5-HT_{2C}-knock-out mice) also augmented the effects of SSRIs on extracellular serotonin levels in the brain. Antagonism of the 5-HT_{2C} receptor resulted in a significantly increased antidepressant effect of SSRIs.

The Neurogenesis Hypothesis

Previous assumptions that neurogenesis does not occur in the adult brain appear to be false. In at least two areas, the subgranular layer (SGL) of the hippocampal dentate gyrus and the subventrical zone (SVZ), neural stem cells have been demonstrated to proliferate. It has been proposed that adult neurogenesis could play a role in both the neurobiology and pharmacotherapy of depression.[52,53]

A recent study by Banasr et al.[54] investigated the role of several 5-HT receptor subtypes in serotonin-stimulated neurogenesis. Although the various agonists and antagonists could have been more selective, the study clearly suggests a role for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2B} receptors in adult neurogenesis in SGL and/or SVZ. This might be a confounding factor with augmentation strategies based on the antagonism of these receptor subtypes, with the possible exception of 5-HT_{1B} receptors, which decrease neurogenesis in SVZ upon activation.

The open question is therefore still whether antidepressants need an effect on neurogenesis and the survival of newborn neurons to enable them to exert their antidepressant/anxiolytic effects.

Stress and glucocorticoids have repeatedly been shown to decrease hippocampal neurogenesis.55 Acute and particularly chronic unpredictable stress have also been shown to impair the proliferation of progenitor cells of newly formed cells in male rat brains. Interestingly, there appear to be gender differences in response to chronic stress in rats, in that the same stressor in female rats led to the increased survival of newly generated neurons.[55,56]

The inhibition of 5-HT synthesis influences neurogenesis, whereas 5-HT_{1A} and 5-HT_{2A} receptor antagonists reduce the number of dividing cells.[57] A number of compounds, such as 5-HT_{1A} agonists that possess anxiolytic and (albeit) weak antidepressant effects in patients, enhance the formation of new cells in the hippocampal area.[58,59] In addition, the brain-derived neurotrophic factor (BDNF) has positive effects on the survival of newly born neurons.[60,61]

A variety of other molecules play a role in neurogenesis, including epidermal growth factor (EGF), insulin-like growth factor (IGF) and transforming growth factor-alpha (TGF-α).[62] As so many different influences may have an impact on the proliferation and survival of newborn cells, more research is needed to disentangle various molecular processes involved in neurogenesis in order to establish its exact role in antidepressant therapy.

Neuropeptides

Neuropeptides are able to modulate monoaminergic transmission in a regionally specific manner, leading to their potential to augment the effects of SSRIs in differential brain areas. Although there are numerous candidates, the discussion considers substance P (SP), corticotropin releasing hormone (CRH), oxytocin (OXT) and arginine-vasopressin (AVP).

Substance P

It has been suggested that SP concentrations and neurokinin 1 (NK_1) receptor densities are altered in depression.[63–65] Furthermore, a study in socially stressed tree shrews demonstrated that both the NK_1 receptor antagonist L-760735 and clomipramine were able to normalise several neuroplasticity parameters.[66] It is noteworthy that the NK_1 receptor antagonists, when combined with an SSRI, augment 5-HT release in mice by modulating substance P/5-HT interactions in the dorsal raphe nucleus.[67] Unfortunately, the outcome of clinical trials was disappointing, which has prompted several pharmaceutical companies to discontinue development of NK_1 receptor antagonists.[68]

Corticotropin-releasing Hormone

Antidepressants have a common trait – they restore the negative feedback between corticosteroids and the HPA-axis, possibly by increasing corticosteroid receptor gene expression. Notably, the CRH_1

receptor antagonist R121919 significantly reduced depression and anxiety scores,[69] without a faster onset of action than regular antidepressants.

CRH is also a modulator of neuronal activity in other brain areas – the question now arises of whether there is a role for the augmentation of SSRIs with CRH receptor antagonists. Interactions between CRH and 5-HT in dorsal raphe nucleus may be of particular interest in the pathophysiology of depression, as witnessed by the increased CRH immunoreactivity in the dorsal raphe nucleus of depressed suicide victims.[70] Some authors have suggested that combining antidepressants with CRH_1 and NK_1 receptor antagonists might help to individualise and optimise efficacy and minimise side effects.[71]

OXT and AVP

The neuropeptides OXT and AVP are long-acting neuro-modudulators and, in addition to their peripheral functions, they may be involved in stress responses, learning and memory. Furthermore, AVP is co-localised with CRH. In animal models, OXT has anxiolytic effects while AVP has anxiogenic effects. A recent study has shown that the CRH_1 receptor antagonist SSR125543A, the V1b AVP receptor antagonist SSR149415 and the clinically effective antidepressant fluoxetine all reverse chronic mild stress-induced suppression of neurogenesis in mice, probably through increased expression of the cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) in dentate gyrus.[72] Post-mortem studies and clinical studies in depressed patients suggest the involvement of AVP and OXT.[73–76] Interestingly, the acute effects of SSRIs on OXT release are mediated by $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A/C}$ receptors, but not by $5\text{-}HT_{1B}$ and $5\text{-}HT_3$ receptors.[77,78]

Beyond Receptors

The interaction between neurotransmitters and their receptors also involves the regulation of intracellular pathways and second messenger signals within cells, which in turn affects how these systems interact and function. Recently, the cAMP second messenger system has gained particular interest owing to its involvement in antidepressant action and its implication in the pathophysiology of depression.[79,80] Chronic administration of antidepressants induces adaptations of the cAMP pathway at several levels. Increased CREB protein expression, for instance, is associated with cAMP-mediated gene transcription and may be part of the mechanism underlying antidepressant activity.[81–85] In contrast, chronic stress has been reported to decrease CREB-mediated transcription in the brain.[86] The up-regulation of CREB, a stimulus-induced transcription factor, indicates that specific target genes may also be regulated by antidepressant treatments.[87]

BDNF is among the many potential target genes that could be regulated by CRE. Pre-clinical studies have reported the reduced expression of BDNF following stress, yet, more importantly, reduced CSF BDNF levels were also observed in depressed subjects.[90] These findings support a potential role for BDNF in depression and suggest its involvement in the actions of stress and stress-related affective disorders.[91] Furthermore, the neurotrophin intracellular cascade appears to be a common target of antidepressants, independent of their pharmacological profile.[92] Together, these lines of evidence provide new insight into the mechanism of antidepressant action and suggest novel targets regarding the development of therapeutic agents.

Compounds that selectively target the CREB-BDNF pathway could have therapeutic merit. It is beyond the scope of this review to elaborate in detail on all the candidate targets; however, some key possibilities will be highlighted. There are several components of the BDNF intracellular signalling cascade that could serve as a target for drug development, i.e. protein kinase A,[93–95] phosphatidylinositol 3-kinase (PI-3-K),[96] B-cell lymphoma 2 (bcl-2),[97] glycogen synthase kinase 3 (GSK-3)[98] and the mitogen-activated protein kinase (MAPK).[99] Compared with drugs targeted at neurotransmitter receptors, compounds that act at intracellular sites are likely to be less selective, yet the existence of multiple forms of these intracellular components could provide regional specificity, due to their characteristic expression patterns in the brain and divergent regulatory mechanisms. Co-administration of agents with different primary mechanisms may even prove to be effective in patients resistant to conventional antidepressants. An overview of enzymes and proteins involved in the transduction of neurotrophic signals and their cellular actions is given in *Table 1*.

It should be in the interests of pharmaceutical companies to extend their ambitions by developing compounds with the cAMP-BDNF pathway in mind. Serotonin seems to be a good starting point, as many of its receptor subtypes are either directly coupled to the cAMP system or influence this pathway at more downstream sites, such as CREB. The multiple signalling cascades possibly involved are very complex, and due to the large overlap and convergence of these pathways, further research is needed to elucidate which signalling pathways are crucial in the regulation of the pathophysiological events associated with components of mood disorders and which are most relevant for the development of more efficient drugs with fewer side effects.

Table 1: Enzymes and Proteins Involved in the Transduction of Neurotrophic Signals and in Their Cellular Actions

Protein/Enzyme	Function
Ras	MAPK kinase kinase kinase
MEK1	MAPK kinase 1: it is responsible for the dual phosphorylation that activates ERK1 and 2
ERK1 and 2	MAP Kinases: they are involved in the transduction of neurotrophin signals and play important roles in the adult brain modulating synaptic plasticity and neuronal survival. They are activated by MEK1: phospho-ERKs can phosphorylate a broad variety of proteins and transcription factors including microtubule-associated proteins, CREB and Fos.
PI-3-Ks	The PI 3-kinase activities have been shown to be necessary for many different cell regulatory pathways. Their functions are crucial during mitogenesis but also in terminally differentiated cells. In neurons, they have a fundamental role in the neurotrophin-mediated survival.
Protein kinase B (Akt-1)	Serine/threonine kinase downstream the PI-3K. This enzyme plays a pivotal role in the modulation of survival in neurons regulating the activity and expression of several pro- and anti-apoptotic proteins, including bcl-2, p-53 and GSK-3-β.
p53	p53-protein functions include maintenance of DNA stability, control of mitochondria integrity and regulation of apoptosis. p53-mediated apoptosis may involve multiple mechanisms, including the interference with the growth factor-mediated survival signals. The inhibition of p53 functions and expression appears to prevent apoptosis.
bcl-2	bcl-2 proteins are involved in the regulation of cell death. Many of these proteins show widespread expression and are also expressed in the nervous system in developing and adult organisms. A physiologic role for bcl-2 in neuron survival has been shown: these proteins have been shown to protect neurons from a wide array of toxic insults.
GSK-3	Glycogen synthase kinase-3-β (GSK-3-β) is a serine/threonine protein kinase ubiquitously expressed and involved in several neuronal functions including cytoskeleton stability and apoptosis. GSK-3-β has been show to be the target of several intracellular cascades. PKA and Akt have been reported to phosphorylate GSK-3-β inhibiting its pro-apoptotic activity.

bcl-2 = B–cell lymphoma 2, CREB = cyclic adenosine monophosphate (cAMP) response element-binding, ERK = extracellular signal-regulated kinase, GSK-3 = glycogen synthase kinase 3, MAPK = mitogen-activated protein kinase, MEK1 = MAPK, PI-3-K = phosphatidylinositol 3-kinase. Source: Trentani et al. (2003).

Other factors that may be involved in the pathophysiology of depression are beyond the scope of this article.[68]

Future Developments

Traditionally, the focus in research has been on neurons, their neurotransmitters and receptor-subtypes, but recent evidence suggests that glial cells in the brain (such as astrocytes) have a much wider range of functions than originally thought, such as synaptic formation and synaptic plasticity. It is now known that the adult central nervous system (CNS) has an impressive regenerative capacity through the mechanism of neurogenesis. Interestingly, there appear to be neurogenic niches in the brain in which astrocytes play a key role.[100] Due to the fact that astrocytes are sensitive to changes in their immediate environment they could be future drug targets, as they may have the ability to restore neurogenesis. In addition, astrocytes involved in synaptic regulation are able to respond to synaptic activity changes by releasing neuro-transmitters, providing bi-directional signalling pathways with neurons in their surroundings.[101] Future research will help to understand exactly how neurotransmitters are released by glial cells and perhaps their potential as targets for new psychotropic drugs.

Conclusions

Although several theoretical questions await further investigation, recent studies indicate that various augmentation strategies are possible in the treatment of depression. Firstly, augmentation with 5-HT_{1A} receptor antagonists remains an interesting but not convincingly proven strategy. Several pharmaceutical companies have now been able to develop an SSRI with potent 5-HT_{1A} receptor antagonistic properties.[102]

Augmentation with 5-HT_{1B} receptor antagonists mght be the most powerful strategy to increase extracellular 5-HT levels, although clinical studies are lacking.

Augmentation with 5-HT_{2C} receptor antagonists is another interesting option. It may not be as potent as 5-HT_{1B} augmentation but it has several advantages compared with the strategies based on antagonism of the classic 5-HT autoreceptors.

The neuropeptides discussed have been associated with either HPA-axis activity, adult neurogenesis or both. Accordingly, antagonists of neuropeptide receptors, with the likely exception of oxytocin receptors, may be suitable partners for SSRIs, in

particular regarding the stress-related symptoms of depression. Finally, a remark is in place concerning the validity of the concept of major depression.

Firstly, several previously described studies suggest that depression cannot be regarded as a single construct. Secondly, in a recent study using time-to-event data from a large epidemiological survey, it was found that for reversible depression the fractional probability of recovery is independent of the preceding history. There is no doubt that life-events may lead to depression in susceptible individuals, although life-events are not single events. The available data suggest that a depressive episode may be the result of a pile-up of negative stimuli and not the result of single life-events.[103] The chronicity of depression may also be a myth people live by. In the same study it was found that 75% of the subjects with depression recover within a year and 50% within three months. Part of the contradictory results in neurobiological studies could be explained by the fact that these patients are included in studies, while in fact it can be questioned whether it is justified to judge these people as being ill. ■

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