

NEUROBIOLOGY OF SUICIDAL BEHAVIOUR

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SUMMARY

It is known that suicidal behaviour has multiple causes. If triggers could be mainly attributed to environmental factors, predisposition could be associated with early stressors on one side such as childhood adversities and genetic predisposition. No convincing animal model of suicide has been produced to date. The study of endophenotypes has been proposed as a good strategy to overcome the methodological difficulties. However, research in suicidal behaviours using endophenotypes entails important methodological problems. Further, serotonergic system was studied in patients with suicidal behaviour primary due to its involvement of serotonin in impulsive-aggressive behaviour, which has been shown to be a major risk factor in suicidal behaviour. Not only on the level of neurotransmitters but also the regulation of neurotropic factors could be impaired in suicide victims. Multiple lines of evidence including studies of levels of BDNF in blood cells and plasma of suicidal patients, postmortem brain studies in suicidal subjects with or without depression, and genetic association studies linking BDNF to suicide suggest that suicidal behaviour may be associated with a decrease in BDNF functioning. It seems that especially specific gene variants regulating the serotonergic system and other neuronal systems involved in stress response are associated with suicidal behaviour. Most genetic studies on suicidal behaviour have considered a small set of functional polymorphisms relevant mostly to monoaminergic neurotransmission. However, genes and epigenetic mechanisms involved in regulation of other factors such as BDNF seem to be even more relevant for further research.

Key words: suicide – genetics – depression – serotonin – neurotransmitters

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INTRODUCTION

On the field of psychiatry and broader, suicidality is a major challenge for today's health care. It was estimated that almost one million deaths each year are attributable to suicide, and suicide attempt is from 10 to 20 times more common than suicide completion (Nocket al. 2008, WHO 2012). Suicidal behaviour defined as the presence of serious suicidal thoughts or ideation or previous suicide attempts is even more common. Further it is estimated that more than 20 million disability-adjusted life-years (years of healthy life lost through premature death or disability) are lost because of suicide worldwide (Levi et al. 2003). It is also estimated that 1.5 million will die from suicide in 2020 worldwide (Levi et al. 2003). Suicide alone represents the 10th leading cause of death worldwide (Levi et al. 2003). The global suicide rate is calculated to around 14 suicides per 100,000 inhabitants. However, the highest annual rates are in Eastern Europe, where 10 countries report more than 27 suicides per 100 000 persons per selected year (WHO 2012). It is known that suicidal behaviour has multiple causes that are broadly divided into proximal stressors or triggers and predisposition (Mann & Currier 2010). If triggers could be mainly attributed to environmental factors, predisposition could be associated with stressors on one side such as childhood adversities and genetic predisposition. However, epigenetic mechanisms could present the bridge between environmental and genetic mechanisms providing additional data on environmental influences on gene expression and transmission of the genetic information from one generation to the next. Beside

factors directly associated with suicidal behaviour, psychiatric illness is a major contributing factor and more than 90% of suicide victims have a psychiatric illness (Lönnqvist et al. 1995). The most prevalent mental disorders analysed by the psychological autopsy method among suicide victims are depressive syndromes (Lönnqvist et al. 1995). Nevertheless, increasing amount of data about suicidal behaviour indicates that suicide is familial and that familial transmission of suicidal behaviour cannot be explained by the transmission of psychiatric disorder alone (Brent & Mann 2005, Mann & Currier 2010). Estimates of heritability for suicide range between 21–50%, and 30–55% for a broader phenotype of suicidal behaviour and ideation (Voracek & Loibl 2007). Substantial evidence from family, twin, and adoption studies corroborates implication of genetic and environmental factors, as well as their interactions, on suicidal behaviour (Mann & Currier 2010). Studies of the neurobiology of suicidal behaviour have become an important and integral part of psychiatric research and encompass techniques from animal models to genetic studies.

ANIMAL MODELS

Animal models are used in the neurobiology research to investigate the etiology, the course and the potential treatment of an illness or behaviour. Although, no convincing animal model of suicide has been produced to date, and despite the intensive study of thousands of animal species naturalists have not identified suicide in nonhuman species in field situations (Preti 2011). However, in some species the

decreased food intake after stressful situation could be viewed as a model of suicidal behaviour. When modelling suicidal behaviour in the animal, the greatest challenge is reproducing the role of will and intention in the neurobiological process of suicidal behaviour (Preti 2011). However, it is possible to use animal models in the investigation of isolated neurobiological systems involved in the suicidal behaviour in humans, such as the cortisol social-stress response and the aggression/impulsivity trait, involving the serotonergic system (Preti 2011, Pandey 2011).

ENDOPHENOTYPES

Endophenotypes are a product of the expression of specific genes involved in a more complex pathophysiological process, which constitutes mental disease or suicidal behaviour (Jimenez-Treviño et al. 2011). The study of endophenotypes has been proposed as a good strategy to overcome the methodological difficulties derived from the nosology in psychiatry, up to the point that it has now been demonstrated to be effective in genetic research of complex psychiatric diseases such as schizophrenia (Gottesman & Gould 2003). The concept of endophenotypes has been proposed by Gottesman and Gould, who established five criteria that should be fulfilled by the endophenotypes in genetic psychiatry: 1. the endophenotype is associated with the disease in the general population 2. the endophenotype is inheritable 3. the endophenotype is a marker of stable trait, independent of the disease status 4. the endophenotype and the disease co-segregate in the family 5. the endophenotype is manifested in unaffected relatives with greater frequency than in the general population (Gottesman & Gould 2003). However, research in suicidal behaviours using endophenotypes entails important methodological problems, both in regards to the definition of the endophenotype as well as to the heterogeneity of the measurements used (Jimenez-Treviño et al. 2011). It was concluded that the results regarding endophenotypes and suicidal behaviour obtained up to date have not met the expectations (Jimenez-Treviño et al. 2011).

GENTIC MECHANISMS

Serotonergic system

Impulsive-aggressive traits are among other risk factors for suicidal behaviour (Brent et al 1999). Serotonergic system was studied in patients with suicidal behaviour primary due to its involvement of serotonin in impulsive-aggressive behaviour, which has been shown to be a major risk factor in suicidal behaviour (Mann et al 2006). Studies of the serotonergic system in suicidal behaviour initially focused on the determination of serotonin (5-hydroxytryptamine, 5HT) metabolites such as 5-hydroxyindoleacetic acid (5HIAA) in the cerebrospinal fluid (CSF) of patients with depression and/or suicidal behaviour. An important

observation was made that depressed patients with suicidal behaviour had lower level of 5HIAA in the CSF and lower levels of serotonin uptake and serotonin transporter in the platelets of patients with suicidal behaviour compared with depressed patients without suicidal behaviour or control subjects (for review see Pandey 2011). Meta-analyses of prospective biological studies of suicide and cerebrospinal fluid 5-HIAA in mood disorders using the penalized quasi-likelihood (PQL) and bootstrap method yield odds ratios for prediction of suicide of 4.48 (Mann et al. 2006). Not only on the levels of metabolites but also other components of the serotonergic system in the central nervous system are dysregulated in suicide victims, indeed less serotonin transporter mRNA and fewer binding sites in the serotonin raphe nuclei were observed (Huang et al. 2004). No statistically important differences between genotypes of controls and suicide victims group as well as no differences in allele distribution were reported for serotonin transporter gene promoter (5-HTTLPR) and intron 2 (VNTR) polymorphisms by other authors (Pungercic et al. 2006). Abnormalities of the serotonergic system were observed in suicide victims in the population with high suicide rates (Videtič et al. 2000). It seems that genetic polymorphisms involved in the regulation of serotonergic systems could influence the response to stress in some individuals. One of the most abundant serotonin receptors in the mammalian brain the autoreceptor 1A (5-HT_{1A}) was beside 2A and 1B receptor subtypes extensively studied in suicide victims (Pandey 2011). Increased 1A receptor production could enhance the negative feedback inhibition of serotonergic Raphe neurons and lead to a lower serotonergic neurotransmission, causing a predisposition to depression and suicidal behaviour (Alber et al. 2011). The observation that the G allele at the position -1019 in promoter of the HTR1A receptor gene increase the expression of this gene, and therefore reduces serotonergic neurotransmission that might predispose to suicidal behaviour. Promoter polymorphism -1019C>G on suicide victims and controls all of Slovenian origin was analysed taking into account stressful life events in a month prior to suicide (Videtič et al. 2000). Differences in the distributions of genotype and allele frequencies were not significant between suicide victims and control group. However, more stressful life events in the month prior to the suicide were reported for the subgroup with CC genotype in comparison to subgroup with CG/GG genotypes (Videtič et al. 2000). However, the results of 5HT_{1A} receptors studied in suicide victims appear to be inconsistent and mixed; an increase in 5HT_{1A} receptors in some cortical areas is reported by some authors but not by others (for review see Pandey 2011). Nevertheless, it does appear that alterations in 5HT_{1A} receptors maybe associated with pathophysiology of suicide in association with mental disorder (Pandey 2011, Albert et al. 2011). Beside 5-HT_{1A} receptor, most studied in respect to suicidal behaviour is the serotonin transporter

5' functional promoter variant, serotonin 5-HT_{1B} and 5-HT_{2A} receptors, and monoamine oxidase A (Rujescu et al. 2007, Mann & Currier 2010). It was summarised that the studies of 5HT_{2A} receptors strongly indicate that increase in the binding and/or protein and mRNA expression of 5HT_{2A} receptors associated with the pathophysiology of suicidal behaviour (Pandey 2011).

Further it is known that tryptophan-hydroxylase (TPH) is the key enzyme in biosynthesis of serotonin (Arango et al. 2003). Two isoforms of TPH has been discovered, namely TPH1 and TPH2. TPH2 was only found in central nervous system, mostly in the brain stem. It was reported that different haplotypes of TPH2 could be risk factors for suicidal behaviour (Zill et al. 2004). Interestingly, in the DNA sequence for TPH2 a very rare functional polymorphism that changes amino acid arginine to histidine (Arg441His; in the DNA 1463 G>A) has been determined with 80 per cent lower function of the enzyme (Zhang et al. 2005). However, association between suicide and aforementioned polymorphism was not determined in a group of suicide victims because this SNP was not polymorphic in studied population (Zupanc et al. 2011). However association was found with another studied SNP (Rs1843809) with less known impact on the enzyme function (Zupanc et al. 2011).

The phosphoinositide (PI) and adenylyl cyclase (AC) signalling systems have been widely studied and implicated in the pathophysiology of suicidal behaviour (Pandey 2011). The regulation of molecules such as G proteins, phospholipase C, protein kinase A, protein kinase C and transcription factor CREB involved in these signalling pathways was studied in suicide victims and patients with suicidal behaviour (for review see Pandey 2011 and Mann and Currier 2010).

Neurotrophins

In general neurotrophins promote the growth and development of immature neurons and enhance the survival and function of specific neuronal populations, including neuronal growth, plasticity, phenotype maturation, synthesis of proteins, and synaptic functioning (Altar et al. 1997, Bartrup et al. 1997, Thoenen 1995, Russo-Neustadt 2003, Sher 2011). For example, brain-derived neurotrophic factor (BDNF) influences a variety of neural processes during the development such as: neurogenesis, neuronal survival and maturation of neural development pathways (Russo-Neustadt 2003). Neurotrophins have the potential to influence not only brain function but also to change brain structure. Indeed it is known that neuronal activity plays a pivotal role in synaptic plasticity and that neurotrophins are potent factors for synaptic modulation. Multiple lines of evidence including studies of levels of BDNF in blood cells and plasma of suicidal patients, postmortem brain studies in suicidal subjects with or without depression, and genetic association studies linking BDNF to suicide suggest that suicidal behaviour may be associated with a decrease in BDNF functioning (Sher 2011). The BDNF

Val66Met variant is a known functional polymorphism (rs6265), consisting of the substitution of valine with methionine in codon 66. It has been shown that the Met allele is associated with the reduced BDNF activity (Egan et al. 2003). Further, a recent meta-analysis including 12 studies showed a trend for the Met-carrying genotypes and Met allele conferring risk for suicide (Zai et al. 2011). Among included studies the study with the largest sample size indicated that the combined Met/Met and Met/Val genotypes of the BDNF Val66Met variant could be the risk factor for violent suicide in female subjects and for suicide in victims exposed to childhood trauma (Pregelj et al. 2011). Also genes regulating other systems could be involved in suicidal behaviour. Abnormalities in the hypothalamic adrenal (HPA) axis in suicidal behaviour and also major depression were reported (Mann & Currier 2010). Further the HPA axis has bidirectional relationships with the serotonergic and noradrenergic systems contributing to the complexity of the neurobiology of suicidal behaviour (Mann & Currier 2010).

Stress response systems

Abnormal stress response has been documented in both the HPA axis and noradrenergic system in suicidal behavior in the context of depression (Mann & Currier 2010). Meta-analyses of prospective biological studies of suicide and the dexamethasone suppression test (DST) in mood disorders using PQL and bootstrap method yield odds ratios for prediction of suicide of 4.65 indicating the dysfunction of the system responsible to stress in suicide victims (Mann et al. 2006). Suicide victims with depression have fewer norepinephrine neurons in the locus ceruleus and, greater beta-2 adrenergic cortical receptor binding, and lower alpha-adrenergic cortical binding have been reported (Mann & Currier 2007). However, the results of studies of alpha- and beta-adrenergic receptors in the postmortem brain of suicide victims still appear to be mixed. Some investigators found an increase in beta-adrenergic receptors, while others did not (for review see Pandey 2011). The metabolism of neurotransmitters could also be impaired in suicide victims by the altered function of the enzymes involved in the degradation of neurotransmitters. Catecholamine degradation is regulated with the activity of the enzyme catechol-O-methyl-transferase (COMT), which catalyses the transfer of a methyl group to catecholamines and degrades dopamine, noradrenaline and adrenaline. A common functional polymorphism (COMT Val108/158Met), a G to A nucleotide transition that results in amino acid substitution from valine (Val) to methionine (Met) on position 158 in the membrane-bound or in position 108 in the soluble form of COMT (rs4680) is known. The Met form is associated with the low-activity of COMT, while the Val form of COMT is related to a high-activity (Kia-Keating et al. 2007). It has been observed that Met/Met genotype leads to a threefold to fourfold decrease in COMT enzymatic activity compared to Val/Val genotype (Kia-Keating et al. 2007). Data on the role of the COMT

Val108/158Met polymorphism and suicidal behaviour are inconclusive (Russ et al. 2000, Calati et al. 2010) and recent meta-analysis suggested that COMT variants may not be directly implicated in suicidal behaviour (Calati et al. 2010). However, similarly to some previous reports (Nolan et al. 2000, Ono et al. 2004), even more recently, differences in the frequency of the Met/Met genotype were observed between in male control subjects and male suicide victims, suggesting that this genotype of the COMT Val108/158Met might be a protective factor against suicide (Pivac et al. 2011).

EPIGENETIC MECHANISMS

Epigenetic studies have the potential to improve our understanding of the etiology of mood and anxiety disorders and suicide by bridging the gap in knowledge between the exogenous environmental exposures and pathophysiology that produce common mood and anxiety disorders and suicide (El-Sayed et al. 2012). Epigenetic modifications are those potentially reversible, mitotically heritable alterations in genomic expression that occur independent of changes in gene sequence (Henikoff & Matzke 1997). Further, epigenetic mechanisms could be defined as the regulation of various genomic functions, including gene expression, which are not based on DNA sequence but rather controlled by heritable and potentially reversible chemical modifications of DNA molecule and/or the chromatin structure (Yanagimachi 2002, Robertson 2002). DNA methylation such as cytosine methylation at the 5' position of the pyrimidine ring is a relatively stable covalent modification of the DNA and is regulated by different types of DNA methyltransferases (DNMTs). There are several DNMTs in the methyltransferase family such as DNMT1 the main enzyme responsible for the maintenance of DNA methylation. From preclinical research it is also known that this enzyme methylates hemimethylated DNA more rapidly than unmethylated DNA (Stein 1982, Yoder et al. 1997) providing the possibility that methylation profiles could be inherited from mother to daughter cell (Li 2002). This transmission from one generation of cells to another is called the epigenetic inheritance system (Maynard & Smith 1990). However, in comparison to nucleotide sequences, the degree of mitotic fidelity of epigenetic patterns is lower (Riggs et al. 1998). Newer investigations suggested that some epigenetic signals do survive gametogenesis and that this information can be passed on from one generation to the next (Rakyan et al. 2001). Epigenetic modifications associated with suicide and common mood and anxiety disorders was reviewed and found out that several studies focused on epigenetic regulation of amine, glucocorticoid, and serotonin metabolism in the production of common mood and anxiety disorders and suicide (El-Sayed et al. 2012). For example, one of the studies included in the review observed an increase of DNA methylation at specific CpG sites in BDNF promoter/exon IV in postmortem brain samples from suicide victims in comparison with

nonsuicide control subjects (Keller et al. 2010). It was also observed that most of the CpG sites lying in the -300/+500 region, on both strands, had low or no methylation, with the exception of a few sites located near the transcriptional start site that had differential methylation, while genome-wide methylation levels were comparable among the subjects (Keller et al. 2010). Interestingly, the observed mean methylation degree at the 4 CpG sites analysed by pyrosequencing was reported to be lower in control subjects than in suicide victims (Keller et al. 2010). It is also known that higher methylation degree corresponded to lower BDNF messenger RNA levels. However, it is not clear whether observed differences among groups are directly related to suicide or to other factors such as childhood abuse. Indeed it was reported that epigenetic mechanisms may play an important role in the interplay between stress exposure such as childhood adversities and genetic vulnerability. In preclinical studies it was first suggested that epigenetic mechanisms may be involved in the modulation of gene expression in response to stressful stimuli. Later, epigenetic differences in a neuron-specific glucocorticoid receptor (NR3C1) promoter between postmortem hippocampus obtained from suicide victims with a history of childhood abuse and those from either suicide victims with no childhood abuse or controls were found, indicating the involvement of these mechanisms in human adaptation to stress (McGowan et al. 2009). On the other hand, beside DNA methylation, histone modulation is involved in epigenetic regulation of gene expression by regulation of diverse chromatin-templated processes, including transcription. In association with DNA molecule nuclear proteins histones are the basic building units of nucleosomes. DNA molecule is wrapped around a protein octamer made of histones. It is also known that each of the histones has a "tail" protruding out of the nucleosome, which can be modified in different ways such as phosphorylated, ubiquitinated, sumoylated, acetylated, and methylated (Vaquero et al. 2003). This modification influence gene transcription (Tate et al. 1993). Beside that methylated cytosines in transcription factor-binding sites change the affinity of DNA for the transcription factor, which in turn alters the transcriptional activity of a gene, methylated cytosines also attract methyl-CpG-binding protein, which recruit chromatin-remodeling proteins e.g. to deacetylate the histones, resulting in transcriptional silencing (Comb & Goodman 1990). Aberrant epigenetic regulation (epimutations) could have similar effect as DNA mutations because an epimutation could lead to the abnormal expression of a gene by enhancing or silencing that gene (McGowan et al. 2009).

MENTAL PAIN

Pain can be dichotomously divided into psychological and physical pain. However, research indicates that on one hand physical pain perception could be influenced by psychological factors and on the other hand psychological factors could produce states in

which physical pain is expressed without any known painful stimulus (Melzack & Wall 1965, Tracey et al. 2000). Mental pain is a hitherto neglected symptom in the study of depression, which according to DSM-IV is strongly linked with suicide. It seems that psychological pain could be independently to depression related with suicidal behaviour (Pregelj 2011). Levels of mental pain measured by regional cerebral blood flow with single photon emission computed tomography were significantly and positively associated with suicidal ideation and levels of hopelessness in a group of depressed individuals (van Heeringen et al. 2010). When compared with patients with low levels of mental pain, those with high levels of mental pain showed relatively increased perfusion in the right dorsolateral prefrontal cortex, occipital cortex and inferior frontal gyrus and in the left inferior temporal gyrus, and relatively decreased perfusion at the medulla (van Heeringen et al. 2010). It was recently reported that recall of suicidal episodes, that is, mental pain plus suicide action, compared to neutral activity, was associated with deactivation in the prefrontal cortex (Reisch et al. 2010). Further they observed that recall of suicide action compared to mental pain, was associated with increased activity in the medial prefrontal cortex, the anterior cingulate cortex, and the hippocampus (Reisch et al. 2010).

CONCLUSIONS

It seems that especially specific gene variants regulating the serotonergic system and other neuronal systems involved in stress response are associated with suicidal behaviour. Most genetic studies on suicidal behaviour have considered a small set of functional polymorphisms relevant mostly to monoaminergic neurotransmission. However, genes and epigenetic mechanisms involved in regulation of other factors such as BDNF seem to be even more relevant for further research.

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