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The role of nitric oxide (NO) donors in anxiety. Lights and shadows

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ABSTRACT

Anxiety-related disorders are a common public health issues. Current medication for this affective disorder involves the GABA-ergic or the serotonergic transmission. Different forms of anxiety, however, are resistant to treatment with GABA-ergic or serotonergic agents and the use of these compounds can be associated with severe side effects. Thus, almost 60 years after the discovery of the benzodiazepines there is need not only for fresh medications but also alternative targets. The nitrergic system has emerged as a promising target since several lines of evidence suggest that nitric oxide (NO), an intra- and inter-cellular messenger in the brain, is implicated in anxiety. Therefore, NO modulators might be beneficial. Here I critically review advances in research of agents acting on the nitrergic system, such as the NO donors, for the treatment of anxiety. Present analysis suggests that although NO donors are involved in anxiety their potential anxiolytic effect remains to be established.

1. Introduction

Anxiety is defined as an adaptive psychological, physiological, and behavioural state that facilitates coping when confronted with an actual or potential threat [1]. Anxiety may become a pathological state, however, and interfere with coping. Anxiety disorders including generalized anxiety disorder (GAD), specific and social phobias, post-traumatic stress disorder (PTSD) obsessive-compulsive disorder (OCD) and panic disorder are a major public health issue worldwide. In this context, the outcome of a series of epidemiological studies suggests that anxiety disorders have the highest lifetime prevalence estimates (13.6–28.8%) and the earliest age of onset (11 years) of psychiatric disorders [2–4].

To date, anxiety disorders have been treated with medications that target γ-aminobutyric acid (GABA) and serotonergic neurotransmission, like benzodiazepines, partial agonists of the serotonergic 5-HT_{1A} receptor and selective serotonin reuptake inhibitors (SSRIs). Some forms of anxiety, however, are relatively resistant to treatment with these agents [5,6]. In addition, either benzodiazepines or SSRIs can be associated with severe side effects, such as sedation, memory deficits, dependence and withdrawal, sexual dysfunction and weight gain. Further, the 5-HT_{1A} receptor partial agonist buspirone has a somewhat limited use, although it is generally well tolerated with few side effects, its efficacy, is less and onset of action slower than previous drugs such as the benzodiazepines [7].

Thus, there is an urgent need to develop alternative treatment strategies [8]. In this context, the nitrergic system has emerged as a promising target since experimental evidence indicates that nitric oxide (NO) is involved in anxiety. Therefore, compounds targeting NO might be beneficial. I discuss the therapeutic potential of agents acting on the nitrergic system such as the NO donors, for anxiety disorders.

2. Nitric oxide (NO)

NO, a short-lived, soluble, highly diffusible gas, is considered an intra- and inter-cellular messenger in the brain [9]. It was identified as an endothelium-derived relaxing factor involved in blood vessel relaxation [10]. It acts in a variety of physiological processes, including cellular immunity [11], vascular tone [12] and neurotransmission [9].

2.1. Synthesis of NO

The conversion of L-arginine to L-citrulline results in NO. This reaction requires oxygen (O_2) and nicotinamide adenine dinucleotide phosphate with flavin adenine dinucleotide, flavin mononucleotide, heme, thiol and tetrahydrobiopterin as cofactors [13]. NO synthase (NOS) is the enzyme required for the synthesis of NO, and three NOS isoforms encoded on different distinct genes have been identified: neuronal NOS (nNOS, NOS-1) which is found in neuronal tissue, inducible NOS (iNOS, NOS-2) whose synthesis is induced by pro-inflammatory factors (cytokines or endotoxin) and endothelial NOS (eNOS, NOS-3), expressed in endothelial cells [14]. nNOS and eNOS are constitutively expressed and require calcium (Ca^{2+}) and calmodulin for their function while the activity of the iNOS isoform is Ca^{2+}-calmodulin

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independent [15].

NO is synthesized after activation of the NMDA receptor. Then Ca^{2+} is transiently rises in the cytosol, forming a complex with calmodulin that binds to and activates nNOS [13]. Glial cells (astrocytes and microglia) generate NO after transcriptional expression of the iNOS isoform [16]. While classic neurotransmitters are water-soluble and cannot cross lipid membranes, NO is both water- and lipid-soluble, so it can diffuse freely to adjacent neurons and act directly on intracellular components from cell to cell [17].

2.2. Main physiological targets of NO

Soluble guanylyl cyclase (sGC), is required for most of NO effects [18]. Its activation produces cyclic guanosine monophosphate (cGMP) when NO binds to a heme group in the enzyme [19]. cGMP then activates a cGMP-dependent protein kinase (PKG) which phosphorylates different proteins. cGMP can also directly activate protein kinases such as the cyclic adenosine monophosphate (cAMP)-dependent kinase (PKA) [20]. Breakdown of cGMP by phosphodiesterase terminates NO/sCG signaling [21]. Therefore, like many conventional neurotransmitters, NO acts through second messengers and activates protein kinases which in turn can affect transcriptional factors and protein synthesis [22].

However, sGC might not be the only physiological target for the biological effects of NO [23], and alternative sGC-independent mechanisms have been described. One reaction of high importance is *S*-nitrosylation of thiol groups of proteins [23–25]. Specifically, the thiol side chains of cysteine residues in proteins can be modified by the addition of an NO group and this outcome could occur by two known routes: the thiol group can be oxidized to a thyl followed by addition of NO, and this reaction is known as oxidative nitrosylation. Alternatively, NO can react with O_2 to form N_2O_3 which then interacts with the thiol group to produce nitrosothiol, and this process is known as nitrosation [24,25].

Depending on the protein species, *S*-nitrosylation can either lower or raise NO activity. The cGMP-independent mechanisms by which NO may exert its biological effects involve three cation channels opened by *S*-nitrosylation, the cyclic nucleotide-gated (CNG) channels; the large conductance Ca^{2+}-activated potassium (BK_{Ca}) channels; the ryanodine receptor Ca^{2+} release (RyR) channels; and the enzyme mono(ADP-ribosyl) transferase [23].

NO seems important in synaptic plasticity, learning and memory [26,27]. It promotes also neuron survival and differentiation and has long-lasting effects through regulation of transcription factors and action on gene expression [15]. NO acts through these mechanisms and its biological effects are dependent on its local levels. In fact, low NO concentrations seem to be neuroprotective and mediate physiological signaling such as neurotransmission or vasorelaxation while at higher concentrations mediate immune/inflammatory actions which are neurotoxic [15].

NO is also involved in regulating the release of different neurotransmitters. It stimulates acetylcholine release in the basal forebrain and ventral striatum, not directly but by stimulating adjacent glutamatergic neurons [26]. Basal NO concentrations lower and high levels raise, GABA efflux in Ca^{2+}- and sodium (Na^{+})-dependent processes [26]. NO donors stimulated but hemoglobin, an endogenous NO scavenger, inhibited noradrenaline and glutamate release in brain areas such as the hippocampus [28]. In rat striatum and medial preoptic area, NO boosted DA and serotonin release in a cGMP-dependent manner [26,29,30].

Its ability to diffuse through cells means that NO can act across an ample volume and its actions are only limited by scavenging or degradation. It can also act as a retrograde messenger at the synapse, mediating transmission from target neurons back onto the synapse and regulating synaptic plasticity. These features enable NO to signal to any local compartment and to cells that lack synaptic activity or NOS expression [31].

2.3. Neurotoxicity of NO

High NO concentrations mediate neurotoxicity [15,32] and can induce neural death through oxidative stress, generation of reactive oxygen intermediates and impairment of antioxidant systems [33]. NO appears to be involved in neurodegeneration through a non-enzymatic reaction with the superoxide anion (O_2^-), forming peroxynitrite ($ONOO^-$), a highly reactive and an extremely potent oxidizing agent [11]. Other mechanisms of NO toxicity, include DNA damage or glutathione depletion [15].

3. NO and anxiety

The implication of NO in anxiety has been proposed although its role in this psychiatric disease has not yet been fully elucidated [34,35]. There is conflicting evidence regarding whether activating the NO-cGMP pathway leads to increased or reduced anxiety-like behaviour.

3.1. Genetic studies

Up to now, few genetic studies have been carried out aiming to examine the implication of NO in anxiety. In a series of preclinical studies it was found that male mice lacking the gene that encodes NOS-1 (NOS-$1^{-/-}$) expressed abnormal anxiety levels respect to their wild-type (WT) counterparts [36–39]. It has also been reported that male NOS-$1^{-/-}$ mice exhibited an exacerbated anxiety-related response in relation to control NOS-$1^{-/-}$ female and control WT mice indicating thus that NO might affect anxiety behaviour in a sex-dependent manner [40]. Further, loss of NOS-1 increased anxiety-like behaviour in the zebrafish and this effect seems to be modulated by serotonin [41].

Studies conducted in humans support a role of polymorphisms in nNOS gene as a risk factor for anxiety disorders [for review see [42]]. In this context, it has been reported that short allele carriers of the functional NOS-1 exon 1f VNTR polymorphism expressed higher anxiety levels than carriers of the long alleles [43,44] eliciting thus an association for NOS-1 with anxiety. Further, a rare mutation in the NOS-1 adaptor protein gene (NOS-1AP) within the coding region for the phosphotyrosine-binding domain, in two siblings with OCD has been revealed [45]. Similarly, it has recently been shown that two genes of the NO pathway, NOS-1AP and NOS-1 seem be involved in PTSD. More specifically, the GG genotype of NOS-1AP polymorphism rs1074489 was associated with PTSD severity [46].

The above described genetic findings appear to propose a role of nNOS in anxiety. There is no information, however, regarding the implication of the other NOS isoforms eNOS and iNOS in this psychiatric disorder. In addition, it is not yet clarified if the effects of NO on anxiety are sex-dependent. Thus, further research is required aiming to address these important issues.

3.2. Histochemical and pharmacological studies

Consistent experimental evidence indicates that nNOS is enriched throughout the limbic system an area important for emotional behaviour [19,32]. In particular, it has been demonstrated that neurons expressing NOS are located in brain areas involved in anxiety such as the dorsolateral periaqueductal grey (dlPAG), the hypothalamus, the medial amygdala (MeA) [47] and the hippocampus [48]. Exposure to the elevated plus maze, a procedure assessing anxiety in rodents, leaded to the activation of neurons containing the enzyme NOS in brain regions related to anxiety [49]. Mice deficient in cGMP kinase II, a downstream mediator of cGMP, exhibited an anxiogenic-like behavioural phenotype. This kinase is highly expressed in cerebral cortex, basal forebrain and amygdala regions thought to be involved in anxiety [50].