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The brain GABA-benzodiazepine receptor alpha-5 subtype in autism spectrum disorder: A pilot [^{11}C]Ro15-4513 positron emission tomography study

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ABSTRACT

GABA (gamma-amino-butyric-acid) is the primary inhibitory neurotransmitter in the human brain. It has been proposed that the symptoms of autism spectrum disorders (ASDs) are the result of deficient GABA neurotransmission, possibly including reduced expression of GABA$_A$ receptors. However, this hypothesis has not been directly tested in living adults with ASD. In this preliminary investigation, we used Positron Emission Tomography (PET) with the benzodiazepine receptor PET ligand [^{11}C]Ro15-4513 to measure α1 and α5 subtypes of the GABA$_A$ receptor levels in the brain of three adult males with well-characterized high-functioning ASD compared with three healthy matched volunteers. We found significantly lower [^{11}C]Ro15-4513 binding throughout the brain of participants with ASD ($p < 0.0001$) compared with controls. Planned region of interest analyses also revealed significant reductions in two limbic brain regions, namely the amygdala and nucleus accumbens bilaterally. Further analysis suggested that these results were driven by lower levels of the GABA$_A$ α5 subtype. These results provide initial evidence of a GABA$_A$ α5 deficit in ASD and support further investigations of the GABA system in this disorder.

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1. Introduction

Autism Spectrum Disorder (ASD) is characterised by deficits in social reciprocity, communication impairments, and restricted, repetitive interests and behaviours (World Health Organization, 1993). Recent research suggests an approximate prevalence of 0.6–1.5% in the general population (Baron-Cohen et al., 2009; Schechter and Grether, 2008; Thomas et al., 2011; Zaroff and Uhm, 2011).

Numerous studies have reported differences in brain anatomy and function in individuals with ASD (e.g. see Craig et al., 2007; Ecker et al., 2010; Hallahan et al., 2009), but the underlying molecular basis of the condition remains unclear. This has led to a paucity of treatment targets. At present, therapeutic options for ASD are limited to medications used to alleviate specific symptoms, such as the licensed use of the antipsychotic risperidone for aggression and challenging behaviours (Matson et al., 2011) and selective serotonin reuptake inhibitors in obsessive or repetitive behaviours (King et al., 2009). However, the efficacy of such pharmacotherapy has recently been questioned (McPheeters et al., 2011). There is, therefore, a need for better targeted, ASD-specific treatments, and this will only be possible on the basis of a better understanding of ASD pathophysiology.

ASD is now viewed as a heterogeneous set of disorders, which can be caused by various genetic, epigenetic and environmental factors, but emerging evidence suggests that an imbalance between excitatory glutamate and inhibitory gamma-amino-butyric-acid (GABA) neurotransmission may form a final common pathway in ASD. In particular, defects in GABA transmission, leading to brain hyperexcitability, have been hypothesized to underlie the symptoms of ASD (Pizzarelli and Cherubini, 2011; Rubenstein and Merzenich, 2003; Yizhar et al., 2011).

GABA, the primary inhibitory neurotransmitter in the adult human brain, is synthesised from the excitatory neurotransmitter glutamate, via the action of glutamate decarboxylase (GAD) enzymes, GAD$_{65}$ and GAD$_{67}$. In the central nervous system, GABA

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is produced and released by inhibitory interneurons (Kubota et al., 2011; Tamamaki and Tomioka, 2010).

GABA acts on two main classes of membrane-bound receptors: ionotropic $GABA_A$ receptors (ligand-gated Cl-channels), and metabotropic (G protein-coupled) $GABA_B$ receptors. The $GABA_A$ receptor family is the predominant type in the brain, and is the site of action of drugs such as benzodiazepines and several anaesthetics (Reynolds et al., 2003).

The $GABA_A$ receptor is composed of five subunits arranged around a central pore (Nutt and Malizia, 2001). The subunits are diverse and different combinations of subunits give rise to $GABA_A$ receptors with specific properties. Most brain $GABA_A$ receptors contain α, β and γ subunits in a 2:2:1 stoichiometry (Tretter et al., 1997), although γ can be replaced by δ or ε subunits, and the σ subunit may substitute for the β subunit.

In man, Positron Emission Tomography (PET) has been critical in delineating the distribution and levels of $GABA_A$ receptors in a variety of neuropsychiatric disorders including anxiety related disorders, benzodiazepine dependence (Nutt and Malizia, 2001), alcoholism, and epilepsy (Malizia and Richardson, 1995).

Evidence for abnormal $GABA_A$ density in ASD comes from neuropathological studies. Blatt and colleagues reported reduced $GABA_A$ receptors and benzodiazepine binding sites in the hippocampus (Blatt et al., 2001), and the cingulate cortex (Oblak et al., 2009, 2010) of individuals with ASD. Other studies found altered GAD expression in ASD (Yip et al., 2007, 2008) and reduced neuronal cell size and increased cell packing density in GABAergic hippocampal neurons and interneurons, subiculum, entorhinal cortex, amygdala, medial septal nucleus, and mammilary bodies in ASD compared to controls (Kemper and Bauman, 1998).

Converging evidence for a $GABA_A$ involvement in ASD comes from genetic studies. For instance, microduplications of the chromosome 15q11–13 locus have been observed in a proportion of people with ASD (Buxbaum et al., 2002; Cook et al., 1998; McCauley et al., 2004; Menold et al., 2001; Sebat et al., 2007; Shao et al., 2003). This region notably contains the genes coding for the $GABA_A$ α5, β3 and γ3 subunits, and although duplication might be expected to lead to over expression of these receptor proteins, in vitro studies of a human neuronal cell line carrying a 15q duplication showed that this variant actually leads to *reduced* GABRB3 expression (Meguro-Horike et al., 2011).

Abnormal patterns of expression of 15q11–13 locus genes have been reported even in ASD cases without the mutation: in 4 of 8 cases of idiopathic ASD, levels of $GABA_A$ α5, β3 and γ3 were reduced as expression of the maternally inherited copies of these genes predominated (Hogart et al., 2007). Finally, reduced frontal and temporal cortical expression of mRNA in a network of genes highly expressed in GABA interneurons was observed in two samples of ASD (Voineagu et al., 2011).

There is, therefore, mounting evidence implicating the GABA system and in particular $GABA_A$ receptors, generally in limbic areas, in ASD. However, there has only been one study which attempted to directly measure $GABA_A$ receptors in the living brain in ASD. This SPECT (Single Photon Emission Computed Tomography) study reported decreases in $GABA_A$, especially in the frontal cortex in children with ASD (Mori et al., 2011). No study has yet examined adults with the condition.

We (Lingford-Hughes et al., 2002) have previously characterized the benzodiazepine receptor PET ligand [^{11}C]Ro15-4513 (Halldin et al., 1992) in rats and humans and showed that its uptake primarily has a limbic distribution. High levels of uptake were described in the anterior cingulate cortex, hippocampus, insular cortex, septal region and amygdala, with lower levels seen in the occipital cortex and cerebellum than that observed with the traditional PET benzodiazepine ligand [^{11}C]flumazenil (Lingford-Hughes et al., 2002).

These differences are explained by [^{11}C]Ro15-4513 having relative selectivity, namely approximately 10-fold higher affinity for the α5 subtype of the $GABA_A$ receptor compared with the other receptor subtypes in both humans and rodents. In addition, recent refinements in the analysis of [^{11}C]Ro15-4513 PET images have allowed us to further refine the signal, giving some discrimination between the high affinity α5 receptor subtype and the highly abundant α1 receptor subtype (Myers et al., 2012).

Therefore, we decided to use [^{11}C]Ro15-4513 PET to measure α1 and α5 $GABA_A$ subtype receptor levels, with a particular interest in the α5 subtype in limbic regions that have been implicated in ASD: the amygdala, the hippocampus, and the nucleus accumbens. In the light of the previous work discussed above, our primary hypothesis was that, compared to controls, individuals with ASD have a significant reduction in α5 GABA receptor availability in these areas.

2. Methods

2.1. Participants

In this preliminary study, we included three male participants diagnosed with autism, meeting ICD-10 Research criteria, who scored above threshold for ASD in the Autism Diagnostic Observation Schedule (ADOS), and who had an IQ above 80. This IQ criterion was applied to avoid the possible confounding influence of IQ. We compared these individuals to data already acquired from three age-sex matched controls. All volunteers had capacity to consent to participation in the study; capacity was assessed by a qualified psychiatrist. English was the first language of all individuals. See Table 1 for participant clinical and demographic details.

We excluded people with a learning disability (mental retardation); people with diagnosed and treated ADHD, hyperkinesis or Tourette's syndrome. We also excluded people who were taking psychotropic medication with possible effects on the GABA system i.e. antiepileptic drugs, benzodiazepines and antidepressants (Bhagwagar et al., 2004); a history of dependence to alcohol or substances of abuse (except nicotine); a major mental illness; or a medical or chromosomal disorder known to be associated with ASD such as Fragile X Syndrome.

All participants were able to tolerate MRI with no history of claustrophobia or presence of a cardiac pacemaker, other electronic medical implant, or ferromagnetic metal foreign bodies.

This study was approved by the North London Research Ethics Committee 3 (REC reference number 10/H0709/90) and by the Administration of Radioactive Substances Advisory Committee (ARSAC); it was also internally approved by the Institute of Psychiatry and the Maudsley Research and Development Office (R&D2011/014) and the Imperial College London and Imperial College Healthcare NHS trust Joint Research Office (JROHH0151).

2.2. Imaging protocol

The PET tracer [^{11}C]Ro15-4513 was synthesised (Halldin et al., 1992) and purified by reverse-phase high performance liquid chromatography (Phenomenex uLTRACARB 7 ODS, 250 × 10 mm). A bolus of [^{11}C]Ro15-4513 was administered through a cannula in the dominant vein of the antecubital fossa. There were no significant differences in dosimetry between the two groups (healthy mean ± s.d.: 40 ± 5 GBq/μmol in 4.14 ± 0.45 μg of stable ligand; ASD mean ± s.d.: 33 ± 11 GBq/μmol in 5.19 ± 1.9845 μg of stable ligand), compared using Student's *t*-tests (specific activity: $t = 1.05$, $p > 0.3$; stable ligand: $t = 0.899$, $p > 0.4$).

Table 1
Participant demographic and psychometric data.

Group	Participant Number	Gender	Age	ADOS[a]	Full Scale IQ
ASD	ASD1	Male	43	14 (5 + 7 + 2)	117
	ASD2	Male	34	13 (3 + 7 + 3)	123
	ASD3	Male	41	11 (4 + 4 + 3)	127
Control	HC1	Male	40	n/a	n/a
	HC2	Male	39	n/a	n/a
	HC3	Male	37	n/a	n/a

[a] ADOS = Autism Diagnostic Observation Schedule − Revised. Total scores are given first. Subscale scores are given in parentheses as follows: (*Communication* + *Reciprocal Social Interaction* + *Stereotyped Behaviour/Restricted Interests*). Note that ADOS and IQ scores were only available for ASD individuals.