Preliminary evidence for an association between aggressive and hostile behaviour and 3α,5α-tetrahydroprogesterone plasma levels in schizophrenia

Gianfranco Spalletta, MD; Elena Romeo, MD; Giuseppina Bonaviri, MD; Giorgio Bernardi, MD; Carlo Caltagirone, MD; Flavia di Michele, MD

Spalletta, Romeo, Bernardi, Caltagirone, di Michele — Department of Neuroscience, University of Tor Vergata, and the I.R.C.C.S. Fondazione Santa Lucia, Rome; Bonaviri — Department of Mental Health, Frosinone, Italy.

Objective: Because it has been suggested that agents acting on the γ-aminobutyric acid-A ($GABA_A$) receptor complex, such as the neuroactive steroid 3α,5α-tetrahydroprogesterone (3α,5α-THP), may be biologic modulators of aggression, we aimed to measure 3α,5α-THP plasma concentrations in subjects with schizophrenia in order to investigate a possible relation with aggressive and hostile behaviour. **Methods:** Eight outpatients with schizophrenia diagnosed according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV), were included. Aggression and hostility were assessed using the Modified Overt Aggression Scale and the paranoid/belligerence symptom cluster of the Positive and Negative Syndrome Scale. Plasma samples were obtained 1 hour before psychometric assessment and were quantified for 3α,5α-THP using a highly sensitive and specific combined analysis by gas chromatography–mass spectrometry. **Results:** Increased aggressiveness and hostility were associated with increased 3α,5α-THP plasma levels (Pearson $r = 0.72$, $p = 0.043$ and Pearson $r = 0.72$, $p = 0.041$, respectively). **Conclusions:** These preliminary results suggest that the neuroactive steroid 3α,5α-THP may affect aggression and hostility in humans.

Objectif : Comme on a laissé entendre que les agents qui agissent sur le complexe récepteur de l'acide γ-aminobutyrique A ($GABA_A$), comme la 3α,5α-tétrahydroprogestérone (3α,5α-THP), stéroïde neuroactif, peuvent être des modulateurs biologiques de l'agression, nous avons cherché à mesurer les concentrations plasmatiques de 3α,5α-THP chez des sujets atteints de schizophrénie afin d'étudier un lien possible avec l'agressivité et l'hostilité. **Méthodes :** Nous avons inclus à l'étude huit patients en service externe atteints d'une schizophrénie diagnostiquée en fonction des critères du *Diagnostic and Statistical Manual of Mental Disorders*, quatrième édition (DSM-IV). Nous avons évalué l'agressivité et l'hostilité en fonction de l'échelle modifiée d'agression manifeste et de la grappe de symptômes paranoïa/belligérance de l'échelle des syndromes positifs et négatifs. Nous avons prélevé, une heure avant l'évaluation psychométrique, des échantillons de plasma que avons soumis à une analyse quantitative pour déterminer la présence de 3α,5α-THP par spectrométrie de masse et chromatographie en phase gazeuse combinées très sensibles et spécifiques. **Résultats :** Nous avons établi un lien entre l'agressivité et l'hostilité accrues et une élévation des concentrations plasmatiques de 3α,5α-THP (Pearson $r = 0,72$, $p = 0,043$ et Pearson $r = 0,72$, $p = 0,041$, respectivement). **Conclusions :** Ces résultats préliminaires indiquent que le stéroïde neuroactif 3α,5α-THP peut avoir un effet sur l'agression et l'hostilité chez les êtres humains. D'autres études s'imposent toutefois pour clarifier la signification fonctionnelle d'un tel lien.

Correspondence to: Dr. Gianfranco Spalletta, IRCCS Santa Lucia, Laboratorio di Neurologia Clinica e Comportamentale, Via Ardeatina 306, 00179 Roma, Italy; fax 39 06 54225988; g.spalletta@hsantalucia.it

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Introduction

Aggression is caused by a heterogeneous mixture of social, psychologic and biologic factors.[1] Improving our knowledge of the biologic markers associated with aggressive behaviour would be of great importance, given that its consequences are potentially disruptive and that control of aggressive behaviour is of the highest priority for physicians.[2]

Involvement of γ-aminobutyric acid (GABA)-ergic neurotransmission in the neurobiology of aggressive behaviour has often been reported.[3,4] In particular, it has been suggested that agents acting on the $GABA_A$ receptor complex may be biologic modulators of aggression. It has also been observed that low quantities of alcohol or benzodiazepines may heighten aggression in humans as well as in animals, whereas higher doses decrease aggression.[5–8] Thus, the neuroactive steroid 3α,5α-tetrahydroprogesterone (3α,5α-THP), the most potent endogenous positive allosteric modulator of the $GABA_A$ receptor complex, which shares many pharmacologic properties with benzodiazepines[9–11] and alcohol,[12] may play a key role in the modulation of aggressive or hostile behaviour.

Recent investigations demonstrate that the administration of low quantities of 3α,5α-THP in mice increases aggressive behaviour in a dose-dependent way, whereas higher doses of 3α,5α-THP or the coadministration of 3α,5α-THP and alcohol may reverse this effect.[13,14] On the other hand, another preclinical study reported a reduction of aggressivity even with low 3α,5α-THP doses (5–15 mg/kg).[15] Guidotti et al,[16] while measuring brain levels of 3α,5α-THP in a mouse model of protracted social isolation compared with a group of house mice, found a downregulation of 3α,5α-THP that may contribute to the development of a late-adaptation syndrome with anxiety, aggression and decreased responses to GABA-mimetic drugs. Although these results shed some light on the possible biologic mechanisms underlying aggressive behaviour in animals, there are no clinical data on the likely relation between aggressiveness and neuroactive steroid levels.

Considering that aggressive behaviour has been, to some extent, associated with schizophrenia,[17] we thought it would be interesting to investigate its biologic correlates in patients with this mental disorder. Furthermore, a fascinating hypothesis concerning the development of schizophrenia during puberty suggests that this disorder may be related to a disturbance of the balance between inhibitory systems in the anterior basal forebrain, via GABA, serotonin or dopamine, and excitatory systems in response to the flood of reproductive hormones (among them steroids) to the brain throughout the reproductive period.[18] Thus, 3α,5α-THP, which is a reduced metabolite of progesterone, may be involved in the occurrence of schizophrenia.[19] Therefore, we aimed to assess 3α,5α-THP plasma concentrations in subjects with schizophrenia who were taking conventional neuroleptics only, in order to investigate the possible relation with aggressive or hostile behaviour in schizophrenia.

Methods

This study included 8 right-handed subjects (6 men and 2 women) with a diagnosis of schizophrenia according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV),[20] all of whom had been consecutively referred to an outpatient psychiatric clinic. All the women in the study were postmenopausal. The clinicians were free to use haloperidol, chlorpromazine, fluphenazine and thioridazine as conventional neuroleptics. They were free to use any dosage and were blind to the aims of the study. Additional inclusion criteria for subjects were the following: age between 18 and 65 years, no major medical or neurologic illnesses, no previous or present additional psychiatric disorders or substance abuse disorder, and no benzodiazepine treatment in the preceding 2 months. At the assessment point, all patients had been receiving stable doses of the conventional neuroleptics listed earlier by the oral route for at least 1 month. All the subjects gave written informed consent after they had received a full explanation of the procedure of the study.

A trained clinical psychiatrist diagnosed schizophrenia according to DSM-IV criteria using the Structured Clinical Interview for DSM-IV, patient edition (SCID-P),[21] and evaluated the psychopathology by using the Positive and Negative Syndrome Scale (PANSS), a clinical-rating scale that permits the evaluation of the patient's level of state hostility (paranoid/belligerence dimension) by summing the scores on the items suspiciousness/persecution, hostility and uncooperativeness obtained during a 45-minute clinical interview.[22] A second trained psychiatrist assessed the patient's aggressive behaviour by using the Modified Overt Aggression Scale (MOAS).[23] MOAS ratings were based on the behaviour of the patient during the week before the assessment and were obtained from the records made by the parents or caregivers, or both. Before the MOAS rating, parents and/or caregivers were given clear explanations as to how to identify and report patients' aggressiveness. The 2 psychiatrists who compiled the PANSS and the MOAS were kept unaware of the other's findings and were not aware of the patients' 3α,5α-THP plasma levels.

Plasma samples were obtained 1 hour before psychometric assessment at 12 am and were quantified for 3α,5α-THP using a highly sensitive and specific combined analysis by gas chromatography–mass spectrometry after extraction with ethyl acetate and separation by thin-layer chromatography (TLC), as described elsewhere.[24,25] Briefly, about 5000 disintegrations per minute of [^{3}H]progesterone were added to the plasma to monitor recovery. After extraction with 3 × 2 mL of ethyl acetate and separation by TLC (i.e., carbon tetrachloride /methanol [99:1, volume of solute per volume of solvent], cyclohexane/ethyl acetate [3:2, volume of solute per volume of solvent]), 7 pmol/L of progesterone was added to the eluate containing 3α,5α-THP as an internal standard. These eluates were lyophilized and derivatized with heptafluorobutyric acid anhydride. Derivatized steroids were analyzed using a Finnigan Trace (Thermo Electron, San Jose, Calif.) gas chromatograph/mass spectrometer equipped with a capillary column (Hewlett-Packard-35 mass spectrometer: length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm). The derivatized steroids were assayed in the negative ion chemical ionization mode (NCI), and the ion at m/z 474 was selectively

monitored. The recovery of tritiated steroids by means of the TLC separation ranged from 80% to 90%. The detection limit for the steroids studied was about 10 fmol/L.

The person who measured the 3α,5α-THP plasma levels was not aware of the patients' rates of hostility and aggression.

Pearson's correlation coefficient was used to assess the relations between continuous variables. The level of significance was set at $p < 0.05$.

Results

The sociodemographic and clinical characteristics of the patients in the study are indicated in Table 1.

Increased aggressiveness (MOAS score) and state hostility (PANSS paranoia/belligerence symptom cluster) were associated with increased 3α,5α-THP plasma levels (Pearson $r = 0.72$, $p = 0.043$ and Pearson $r = 0.72$, $p = 0.041$, respectively) (Fig. 1).

Furthermore, the neuroleptic dosages, when converted to estimated chlorpromazine equivalents,[26] were positively associated with 3α,5α-THP plasma levels (Pearson $r = 0.56$), but this correlation was not statistically significant ($p = 0.16$).

Finally, the chlorpromazine equivalents were positively correlated with the level of hostile (Pearson $r = 0.49$) and aggressive (Pearson $r = 0.29$) behaviour, but these correlations were not significant ($p = 0.23$ and $p = 0.51$, respectively).

Table 1: Sociodemographic and clinical data of 8 study subjects with schizophrenia

Variable	Mean (and SD)	Range
Age, yr	38.4 (9.6)	28–58
Years of education	8.9 (2.7)	5–13
Age at onset of disease, yr	24.9 (7.8)	19–40
Duration of illness, yr	13.5 (4.8)	7–21
Chlorpromazine equivalent, mg/d	472 (352)	100–1112
3α,5α-THP level, nmol/L	5.01 (3.81)	1.01–11.12
PANSS		
Positive scale	23.0 (4.7)	16–29
Negative scale	24.2 (8.8)	10–33
General psychopathology scale	49.6 (15.1)	26–70
Paranoia/belligerence scale	9.6 (2.4)	6–13
MOAS	7.6 (7.4)	0–20

Note: MOAS = Modified Overt Aggression Scale; PANSS = Positive and Negative Syndrome Scale; SD = standard deviation; 3α,5α-THP = 3α,5α-tetrahydroprogesterone.

Discussion

According to the literature, GABAergic mechanisms seem to be involved in the control of aggressiveness, thereby suggesting a role for 3α,5α-THP in this behaviour. In fact, 3α,5α-THP is the most potent positive allosteric modulator of GABA action at $GABA_A$ receptors in the central nervous system and may serve as an endogenous anxiolytic.[27,28] However, the relation between 3α,5α-THP and aggressive behaviour has only been investigated in preclinical studies, and the results are still controversial.[14–16]

To our knowledge, this is the first clinical study that has aimed to investigate the possible association between 3α,5α-THP levels and the manifestation of aggressive and hostile behaviour in a sample of patients with schizophrenia. Although these data may provide valuable information for a better understanding of the mechanisms of $GABA_A$-receptor regulation underlying aggressive behaviour in schizophrenia, only limited conclusions can be drawn because of the small size of our sample and the concomitant neuroleptic treatment. Furthermore, given the difficulty of recruiting drug-free subjects, we opted to include only patients treated with conventional neuroleptics in this first study, because there is evidence that the atypical antipsychotics may modify brain concentrations of 3α,5α-THP in rats.[29,30]

In reality, contrary to the previously mentioned preclinical study,[30] our results have shown in humans that there is a fairly strong positive correlation even for conventional neuroleptic dosages with 3α,5α-THP levels, although this is not statistically significant. This may indicate the possibility that the higher dosage of antidopaminergic drugs could increase

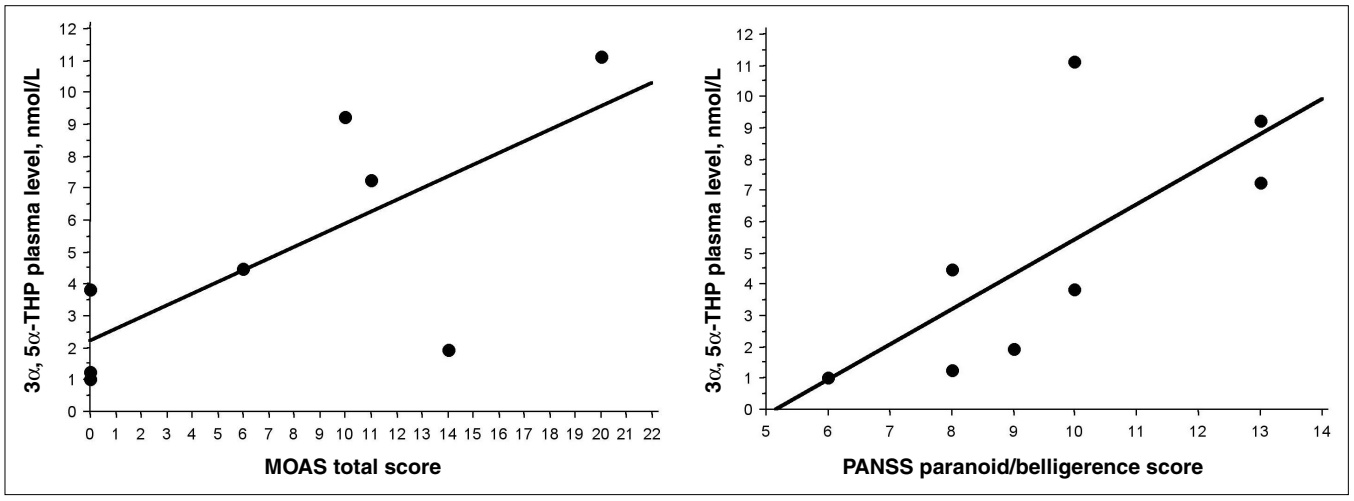

Fig. 1: Relation between scores on the Modified Overt Aggression Scale (MOAS) (left) or the Positive and Negative Syndrome Scale (PANSS) paranoid/belligerence cluster (right) and 3α,5α-tetrahydroprogesterone (3α,5α-THP) plasma level in 8 patients with schizophrenia.

3α,5α-THP plasma levels in the most aggressive patients. An alternative explanation can be found in the well-documented usual tendency of clinicians to increase neuroleptic dosages in the more hostile patients in an attempt to control their behaviour.[31] If this is the case, the link between neuroleptic dosages and 3α,5α-THP plasma levels could be secondary to the relation between neuroleptic dosages and hostile behaviour.

However, the most important result of this study was a strong positive correlation between 3α,5α-THP plasma levels and both aggressive and state hostile behaviour in our sample of patients.

Interestingly, our findings are in line with evidence in mice of increasing aggressivity associated with the intake of low doses of positive modulators of the $GABA_A$-receptor complex, such as benzodiazepines, alcohol and 3α,5α-THP, in a dose-dependent manner.[14,32]

Furthermore, it has been shown that high concentrations of 3α,5α-THP or coadministration of 3α,5α-THP with another $GABA_A$-receptor-positive modulator (such as midazolam or alcohol) may reverse the effect on aggression (suppressing aggression), possibly indicating a complex key role for 3α,5α-THP in the action of GABAergic modulators on postsynaptic $GABA_A$-receptor function.[14,33,34] Therefore, we might expect to find a diminution of aggression in patients with schizophrenia treated with added benzodiazepines, an issue that certainly requires to be investigated further.

In conclusion, the current preliminary result of a positive correlation between 3α,5α-THP plasma levels and aggressive or hostile behaviour suggests that 3α,5α-THP may affect aggression in humans**.** However, more investigations are necessary to clarify the functional significance of this evidence in the pathogenesis of aggressive behaviour in schizophrenia.

Competing interests: None declared.

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