Central serotonergic hypofunction in autism: results of the 5-hydroxy-tryptophan challenge test

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Abstract

Some studies have suggested that disorders in the central serotonergic function may play a role in the pathophysiology of autistic disorder. In order to assess the central serotonergic turnover in autism, this study examines the cortisol and prolactin responses to administration of L-5-hydroxy-tryptophan (5-HTP), the direct precursor of 5-HT in 18 male, post-pubertal, Caucasian autistic patients (age 13–19 y.; I.Q.>55) and 22 matched healthy volunteers. Serum cortisol and prolactin were determined 45 and 30 minutes before administration of 5-HTP (4 mg/kg in non enteric-coated tablets) or an identical placebo in a single blind order and, thereafter, every 30 minutes over a 3-hour period. The 5-HTP-induced increases in serum cortisol were significantly lower in autistic patients than in controls, whereas there were no significant differences in 5-HTP-induced prolactin responses between both study groups. In baseline conditions, no significant differences were found in serum cortisol and prolactin between autistic and normal children.

The results suggest that autism is accompanied by a central serotonergic hypoactivity and that the latter could play a role in the pathophysiology of autism.

INTRODUCTION

Autism is a pervasive developmental disorder. The core symptoms and diagnostic criteria according to the DSM-III-R and DSM-IV [1] are a qualitative impairment in social interaction and language and restricted, repetitive and stereotyped patterns of behavior, interest and activities. The condition is highly prevalent (5–15 per 10 000) and is four times more common in boys than in girls.

Serotonin (5-HT) plays an important role in developmental disorders because of its role as a morphogenetic agent and a differentiation factor in the developing brain and as a neurotransmitter in the CNS [2]. There is some evidence for a role of 5-HT in the pathophysiology of autism. A frequently

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reported finding in autism is the increase in whole blood concentrations of serotonin (5-HT) [3–5].

Selective 5-HT reuptake inhibitors (SSRIs) have beneficial effects in the treatment of patients with autism [6]. Tryptophan depletion techniques result in a significant increase in autistic behaviors [7]. PET-scan studies reveal decreased 5-HT synthesis in frontal cortex and thalamus, but elevated 5-HT synthesis in the contra-lateral dentate nucleus [8]. There is some preliminary evidence of linkage and association between the 5-HT transporter gene and autistic behavior [9,10]. A genetic predisposition for abnormalities in the serotonergic system in autism is also reported by Cook and Leventhal [11] who demonstrated that the hyperserotonemia may be familial. Cook et al. [4] postulated the existence of a normoserotonaemic subgroup with an increased [^{3}H]-paroxetine binding whereas the density of platelet 5-HT2 receptor binding sites was significantely lower in the hyperserotonaemic subgroup. Croonenberghs et al. [12] reported an increase in the platelet [^{3}H]-paroxetine Kd values in autism, suggesting a decreased affinity for 5-HT in the platelet 5-HT transporter system.

Challenge tests with serotonergic agonists, such as 5-HTP, are frequently used to evaluate central serotonergic function. Since the release of hormones, such as prolactin and the hypothalamic pituitary adrenal (HPA)-axis hormones, adrenocorticotropic hormone and cortisol, are mediated in part by 5-HT, these hormonal responses to serotonergic agonists offer an index of 5-HT receptor sensitivity in the hypothalamus and the functional state of the central serotonergic system [13]. The 5-HTP challenge test has frequently been used in depression research whereby 200 mg 5-HTP, in non-enteric coated tablets, reliably stimulates cortisol and prolactin responses in adult humans [13–16]. There is evidence that in rodents and man the increase in plasma cortisol and prolactin following 5-HTP are modulated by at least three different postsynaptic receptors, i.e. 5-HT1A, 5-HT2A and/or 5-HT2C [review: 16]. Only one study, published in Japanese, has examined the 5-HTP challenge test in autistic patients [17]. These authors found a blunted prolactin response to 5-HTP administration in autistic children compared with normal controls. In another serotonergic challenge study it was found that the fenfluramine-induced prolactin responses are decreased in a small sample of young adult males with autism [18]. The above results of neuroendocrine tests may indicate that autism is associated with a central hyporesponsivity in the serotonergic system. Therefore, the aims of the present study were to examine whether the 5-HTP-induced cortisol and prolactin responses are lower in patients with autism as compared with normal controls.

SUBJECTS AND METHODS

Subjects

In the present study, forty male subjects, i.e. 18 autistic youngsters and 22 normal controls, aged between 13 y.–19 y. and with an I.Q.>55 participated. One subject in the autistic group had a mild mental retardation (I.Q. between 55–60), all other subjects showed a borderline intellectual functioning (I.Q. between 71–84) or normal intellectual functioning (I.Q. between 85–120). All subjects passed the onset of puberty (Tanner-stage III–IV) and were of the Caucasian race.

Normal volunteers and autistic patients had a normal hematologic screening. All subjects were free of any infections, inflammatory or allergic reactions for at least 2 weeks prior to the blood samplings. Exclusion criteria for autistic patients and healthy volunteers were: subjects suffering from a neurological, inflammatory, endocrine or clinically significant chronic disease; immunocompromized subjects; subjects with an active seizure disorder; subjects with tuberous sclerosis, FRAXA or other chromosomal disorders; and subjects receiving drugs with known or potential interaction with immune and endocrine functions. All healthy youngsters had a negative past, present or family history for psychiatric disorders such as autistic-, bipolar-, schizophrenic-, paranoid-, organic mental- and eating disorders and psychoactive substance use. None was a regular drinker and none had ever been taking psychotropic drugs. All were free of any medications and substance abuse for at least one month. All subjects had a negative drug-screening in the urine.

Clinical evaluation

The autistic youngsters were recruited from the outpatient clinic of Child and Adolescent Psychiatry in Antwerp, Belgium; the Mental Health agencies of the same city; and from a Residential Treatment Centre for Autistic Youngsters in Booischot, Belgium. We employed the DSM-IV [1] criteria to make the diagnosis of autism. The diagnosis was made on the basis of a consensus between, at least three clinicians (psychiatrists and psychologists), working with the autistic subjects in residential, semi-residential or day-care centers. The Autism Diagnostic Interview-Revised (ADI-R) [19], a semi-structured interview, was performed with the parents of youngsters with autism by a trained Master's level clinician (who was blind to clinical status before the interview) or by the primary author. Consensus meetings were held after the structured interview with the clinician. In order to evaluate the associated behaviors frequently seen in autism and the absence of psychopathology in the control-group, all subjects completed the Youth Self Report (YSR) scale [20] during the initial screening. Parents of subjects completed the Child Behavior Checklist (CBCL) [21] and the Aberrant Behavior Checklist (ABC) [22].

Methods

Subjects were kept at rest during the blood collections. Subjects were not allowed to eat or drink during the study period. In order to control for possible seasonal effects in serotonergic measurements all samples were collected in the same season, i.e. between July and September 2000. Each patient and control was tested on two consecutive

days with administration of 5-HTP or indistinguishable placebo, orally, in a single blind, randomized order. The subjects arrived at the Clinical Research Center around 7:15 a.m. After insertion of an intravenous cannula between 7:45 a.m. and 8.00 a.m., blood collections were carried out for the assay of serum cortisol and prolactin and plasma 5-HTP, i.e. 45 (t-45) and 30 (t-30) minutes before administration of placebo or 5-HTP at t0 and, thereafter, every 30 minutes over a 3-hour period, i.e. t30, t60, t90, t120, t150 and t180. At t0, subjects received either a single oral dose of 5-HTP (4 mg/kg; non enteric-coated) or an identical placebo in a single blind order. Serum cortisol and prolactin were assayed in all serum samples, whereas plasma 5-HTP was assayed in the t0, t60, t120, t150 and t180 samples only. Blood was stored in plastic tubes at –75 °C until thawed for assay. All assays were done blind to the subject's status. In order to minimize the analytical variability, all blood specimens for the assays of the above parameters were assayed in a single run with a single lot number of reagents and consumables employed by a single operator. Cortisol and prolactin were measured using the Bayer Immuno 1 system (Bayer, Brussels, Belgium). The inter-assays coefficients of variation (CV) for cortisol and prolactin are 6.7% and 3.0%, respectively. The intra-assay CV values for cortisol and prolactin are 3.1% and 1.6%, respectively. A HPLC method (Gilson, Namur, Belgium) was employed to measure plasma 5-HTP. The inter-assay and intra-assay CV values for 5-HTP obtained in our laboratory are 6.1% and 4.1%, respectively.

Statistics

The independence between classification systems was checked with the analysis of contingency tables (χ^2 test). Group mean differences were checked by means of analysis of variance (ANOVA). Repeated measure (RM) design ANOVAs or RM design analyses of covariance (ANCOVAs) were employed to examine a) the within-subject variability with the effects of time and drug, i.e. 5-HTP versus placebo; b) the between-subject variability with diagnosis (autism versus controls) as treatment; c) the two way interactions between time × drug; drug × diagnosis; and time × diagnosis; and d) the three way interaction term time × drug × diagnosis. Tests on simple effects were carried out in order to examine significant main effects or significant interaction patterns. Relationships between variables were assessed with Pearson's product-moment correlation coefficients and through regression analyses. Normality of distribution was assessed with the Kolmogorov-Smirnov test. Transformations (natural ln) were used to reach normality of distribution or to adjust for heterogeneity of variance between study groups.

RESULTS

Demographics

There were no significant differences in age, Tanner stage and body mass index (BMI) between patients with autism and healthy volunteers. On the CBCL, autistic subjects showed significantly more thought problems, social problems, and greater withdrawal, somatic complaints, anxiety and depression and attentional problems than controls. The CBCL-total score was significantly higher in the autistic subjects. On the YSR autistic subjects reported significantly more social problems and internalizing symptoms (including greater withdrawal and attentional problems subscale measures).

Examination of the subscales of the Aberrant Behavior Checklist (results not shown) showed that there are no subjects who suffer from irritability (aggression, self-injurious behavior, temper tantrums, irritability, screaming, extreme mood changes). Ten out of the 18 autistic patients show a high score on the social withdrawal subscale (lethargy, inactivity, few social or emotional reactions); 2 out of the 18 subjects present a high score on the stereotypic behavior subscale (repetitive movements, odd in behavior) and 10 out of the 18 subjects present a high score on the hyperactivity subscale (inattentiveness, hyperactivity, impulsive, uncooperative, disturbing others).

5-HTP-induced cortisol and prolactin responses

Figure 1 shows the measurements of serum cortisol during the L-5-HTP/placebo challenge test in autistic patients and normal volunteers. A RM design ANOVA performed on the log-transformed cortisol values from t0 to t180 showed a significant effect of time ($F=6.3$, $df=5/418$, $p=0.00006$) and 5-HTP ($F=106$, $df=1/418$, $p<10^{-5}$) and significant interactions between 5-HTP × time ($F=8.1$, $df=5/418$, $p<10^{-5}$) and 5-HTP × diagnosis ($F=8.2$, $df=1/418$, $p=0.004$), but no significant interaction patterns between time × diagnosis ($F=0.1$, $df=5/418$, $p=0.9$) and time × drug × diagnosis ($F=0.6$, $df=5/418$, $p=0.7$). Analyses of simple effects performed after ANOVA showed i) significant effects of 5-HTP in controls ($F=86.8$, $df=1/418$, $p<10^5$), but less in autistic subjects ($F=27.7$, $df=1/418$, $p=0.00001$); and ii) significantly lower cortisol values in autistic patients then in normal controls after 5-HTP administration ($F=3.8$, $df=1/418$, $p=0.04$), but not in baseline conditions ($F=2.0$, $df=1/418$, $p=0.2$). These results show that the significant interaction between drug × diagnosis found in the RM design ANOVA should be explained by 5-HTP increasing serum cortisol significantly less in autistic subjects than in controls. A RM design ANCOVA with t0 as covariate showed the same results; the drug × diagnosis interaction remained significant ($F=5.1$, $df=1/413$, $p=0.02$).

Figure 2 shows the measurements of plasma prolactin during the L-5-HTP/placebo challenge test in autistic patients and normal volunteers. A RM design ANOVA performed on the log-transformed prolactin data from t0 to t180 showed a significant effect of time ($F=19.8$, $df=5/418$, $p<10^{-5}$) and 5-HTP ($F=11.5$, $df=1/418$, $p=0.001$) and a significant 5-HTP × time interaction ($F=9.7$, $df=5/418$, $p<10^{-5}$), but no significant interactions between time × diagnosis ($F=1.4$, $df=5/418$, $p=0.2$), 5-HTP × diagnosis ($F=0.6$, $df=1/418$, $p=0.5$) and time × 5-HTP × diagnosis ($F=0.8$, $df=5/418$, $p=0.5$). A RM design ANCOVA with t0

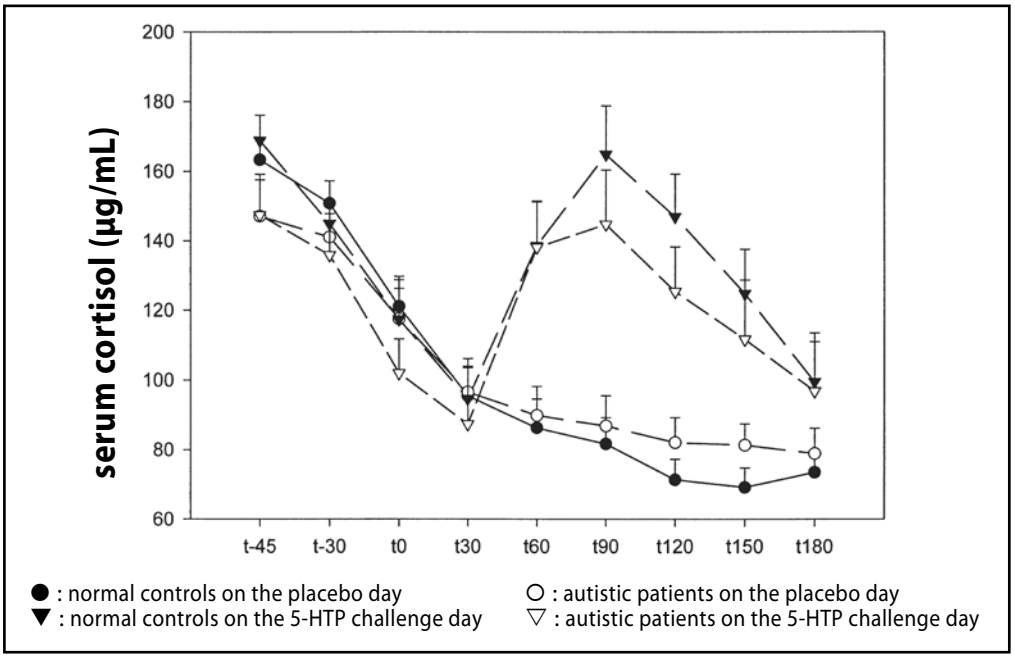

Figure 1. This figure shows the mean plasma cortisol (±SEM) concentrations 45 (t-45) and 30 (t-30) minutes prior to administration of placebo or 5-hydroxytryptophan (4 mg/kg in non enteric-coated tablets) at t0, and 30 (t30), 60 (t60), 120 (t120), 150 (t150) and 180 (t180) minutes later in autistic patients and matched controls.

as covariate showed the same results; the drug × diagnosis interaction was not significant in this ANCOVA (F=2.2, df=1/413, p=0.1).

Regression analyses pooled over the subjects (intra-class correlations) and performed on the data from t0 to t180 show significant time-relationships between the changes in plasma cortisol and prolactin (F=139, df=1/439, $p<10^{-5}$; r=0.49).

Baseline serum cortisol and prolactin

A RM design ANOVA performed on the cortisol values obtained during the placebo day showed that there were no significant differences (F=0.5, df=1/38, p=0.5) in baseline cortisol between autistic children (mean±SD=85.9±33.5 g/mL) and controls (mean±SD=79.5±34.1 g/mL) and no significant interaction pattern between time × diagnosis (F=0.5, df=5/190, p=0.8). A RM design ANOVA performed on the prolactin values obtained during the placebo day showed that there were no significant differences (F=0.3, df=1/38, p=0.6) in baseline prolactin between autistic children (mean±SD=145.5±77.8 U/mL) and controls (mean±SD=143.3±41.7 U/mL) and no significant interaction pattern between time × diagnosis (F=0.9, df=5/190, p=0.5).

Plasma 5-HTP, order effects and age

A RM design ANOVA performed on the 5-HTP values obtained at t0, t60, t120, t150 and t180 on the active challenge day showed a significant effect of time (F=58.7, df=4/152, $p<10^{-5}$) and no significant interaction pattern between time × diagnosis (F=1.1, df=4/152, p=0.4). A RM design ANOVA performed on the 5-HTP values measured on the 5-HTP challenge day at t60, t120, t150 and t180 (the 4 time points at which 5-HTP concentrations were measurable), did not show significant differences (F=2.0, df=1/38, p=0.2) in plasma 5-HTP levels between autistic patients (mean value of the 4 time points=984±547 pg/mL) and controls (mean=1227±673 pg/mL). Regression analyses pooled over the subjects (intra-class correlations) and performed on the data obtained on the 5-HTP challenge day show no significant time-relationships between the changes in plasma 5-HTP and cortisol (F=1.3, df=1/159, p=0.2; r=0.11) or between plasma 5-HTP and prolactin (F=0.0, df=1/159, p=0.99; r=0.0). In order to exclude possible order effects (5-HTP and identical placebo were administered in a single blind order on two consecutive study days) we have carried out additional RM design ANOVAs considering the within-subject (time) and between-subject (order effect) variability. RM design ANOVAs did not show significant interaction patterns between time × order effect either for plasma cortisol (F=1.7, df=5/190, p=0.2) or prolactin (F=1.8, df=5/190, p=0.1). There were no significant correlations between age and cortisol or prolactin at the different time points during the challenge test (even without p-correction).

DISCUSSION

The first major finding of this study is that the 5-HTP-induced cortisol responses were significantly lower in autistic patients then in normal volunteers, whereas there were no significant differences in the 5-HTP-induced prolactin responses between autistic children and controls. Hoshino et al. [17] found a blunted prolactin response after 5-HTP administration in autistic children compared with controls. The study group of Hoshino et al., however, was composed mainly of mentally retarded autistic children. Therefore, it is not clear whether the

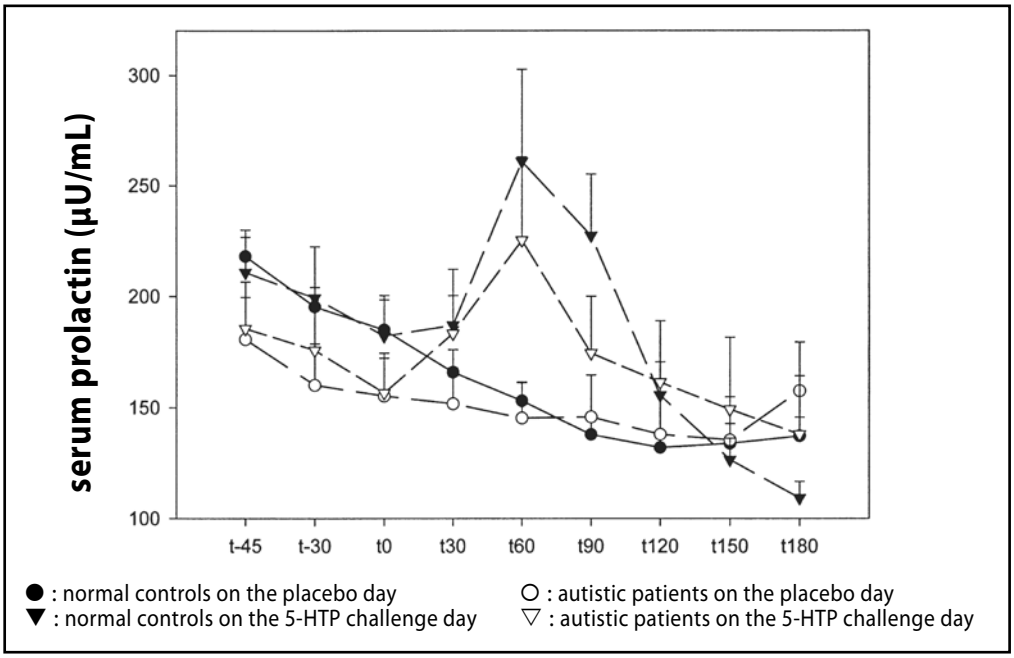

Figure 2. This figure shows the mean plasma prolactin (±SEM) concentrations 45 (t-45) and 30 (t-30) minutes prior to administration of placebo or 5-hydroxytryptophan (4 mg/kg in non enteric-coated tablets) at t0, and 30 (t30), 60 (t60), 120 (t120), 150 (t150) and 180 (t180) minutes later in autistic patients and matched controls.

lowered 5-HTP-induced prolactin responses are related to mental retardation rather that to autism per se. Mc Bride et al. [18] reported a blunted fenfluramine-induced prolactin release in a small sample of young adult males with autism. D,L-fenfluramine promotes a rapid release of 5-HT and inhibits its reuptake, and may function as an indirect 5-HT receptor agonist [23]. In rats and man, fenfluramine produces a dose-dependent increase in prolactin secretion [24]. D-L fenfluramine, however, is not serotonin selective [25] since this drug releases dopamine and noradrenaline and may block striatal dopaminergic receptors [26]. This is important since prolactin release is under dopaminergic control [27]. Thus, a combination of serotonergic and dopaminergic effects could account for blunted fenfluramine-induced prolactin responses. Some authors have also argued that a challenge with 5-HTP is not a selective 5-HT probe since administration of very high doses of 5-HTP in rodents may lead to 5-HT synthesis in central catecholaminergic neurons [28] and increased synthesis of catecholamines [29]. The dose of L-5-HTP used in human studies, however, is much lower than that needed to increase catecholaminergic turnover in animal studies [13].

The results that the 5-HTP-induced cortisol (this study) and the 5-HTP- and fenfluramine-induced prolactin [17,18] responses are diminished in autism may indicate a hyporesponsive serotonergic neuron. These findings could indicate either a presynaptic and/or a postsynaptic hypofunction, the latter being caused by a downregulation or subsensitivity of postsynaptic 5-HT receptors. Our findings extent and strengthen other results that autism may be associated with a central serotonergic hyporesponsivity. For example, Cohen et al. [30–32] found that, after pretreatment with probenecid, the 5-HIAA concentrations in CSF are significantly lower in autistic children than in children with psychotic disorders or mental retardation. As described in the Introduction, evidence that the central serotonergic system may be deficient in autism comes from pharmacological studies with SSRIs [6], behavioral research, such as results of tryptophan depletion techniques [7] and research into the genomics and proteomics of autism [9,11]. Using alpha-[11C]methyl-L-tryptophan as a tracer for 5-HT synthesis with positron emission tomography, Chugani et al. [8] report decreased 5-HT synthesis in the left or right frontal cortex and thalamus, whereas elevated 5-HT synthesis was found in the contralateral dentate nucleus.

The lack of a significant change in the 5-HTP-induced prolactin responses in autism together with a significantly blunted cortisol response may perhaps be explained by differences in 5-HT receptors mediating the prolactin versus cortisol response. There is evidence suggesting that the 5-HTP-induced cortisol and prolactin responses may in part proceed through stimulation of at least three postsynaptic receptors, i.e. 5-HT1A, 5-HT2A and 5-HT2C [review: 33]. Thus, ritanserin, a 5-HT2A/C antagonist, and pindolol, a 5-HT1A receptor antagonist, are able to inhibit the prolactin responses to L-5-HTP in man [33]. In the rodent, high doses of pindolol are unable to inhibit the 5-HTP-induced increase in HPA-axis hormone secretion, whereas ketanserin and ritanserin do [34,35]. In addition, the 5-HTP-induced prolactin release could be dependent upon a functional cooperativity between the 5-HT1A and 5-HT2A/5-HT2C receptors [33]. Thus, depending upon differences in 5-HT1A receptor functioning or in the functional relationship between the 5-HT1A and 5-HT2A/2C receptors between autistic patients and controls, the results of 5-HTP challenge tests

using prolactin may be less clear than those using cortisol as outcome measure. In any case, we may hypothesize that a subsensitivity of the central postsynaptic 5-HT, e.g. the 5-HT2A/2C, receptors may be implicated in the pathophysiology of autism. In order to confirm this hypothesis, future research should focus on 1) neuroendocrine challenge test with more specific 5-HT1A versus 5-HT2A/2C agonists; and 2) SPECT or PET scan studies with specific ligands at these receptor sites. However, many more 5-HT receptors and other aspects of the serotonergic and other neurotransmitter systems may be implicated in the pathophysiology of autism.

Another finding of the present study is that there were no significant differences in baseline cortisol or prolactin values between autistic children and controls. Normal baseline cortisol [36] and prolactin [18] values have been reported in autistic children. Disturbances in HPA-axis functioning have been described in a number of other studies. For example, it has been found that a proportion of autistic children fail to suppress cortisol in response to dexamethasone [36,37]. Since the non-suppressor subjects in these two above studies consisted of lower functioning children, it cannot be ruled out that the findings might be related to the degree of mental handicap and/or organic brain damage. In this respect, Sandman et al. [38] reported that a significant proportion of mentally handicapped adults exhibit a failure to suppress cortisol in response to dexamethasone. Moreover, although there is a strong relationship between baseline plasma cortisol and its responses to dexamethasone, the latter does not solely reflect baseline HPA-axis activity [39].

In our study, we did not find significant differences in plasma 5-HTP concentrations at any of the time points between autistic and normal children, while there were no significant relationships between plasma 5-HTP concentrations and the 5-HTP-induced hormonal responses. Thus, the blunted 5-HTP-induced cortisol response in autism is not related to differences in 5-HTP pharmacokinetics between the study groups.

In the present study, we also measured peripheral measures of serotonergic turnover, such as the plasma levels of 5-HT following administration of 5-HTP [40]. In that study we found i) a 14.5% elevation in baseline whole blood 5-HT in the autistic group, which was not significantly different from that in normal controls; and ii) that 5-HTP administration significantly elevated 5-HT concentrations in autistic children but not in normal controls. These findings were interpreted to indicate that autism is accompanied by an enhanced peripheral metabolism of 5-HT and that serotonergic disturbances in autism, such as the frequently reported hyperserotonemia in whole blood, may be caused by an increased synthesis of 5-HT out of its direct precursor [40]. Schain and Freedman [3] also reported that the urinary concentrations of 5-HIAA are higher in autistic than in mentally retarded children. Thus, it appears that some parts of the central serotonergic turnover may be downregulated in autism, whereas the peripheral turnover may be enhanced. Mc.Bride et al. [18] showed that, in autism, the fenfluramine-induced prolactin responses correlated negatively with whole-blood serotonin concentrations, suggesting a possible relationship between the central and peripheral serotonergic component. There is also a report of a child who had leukodystrophy and had high blood 5-HT concentrations during life, but very low 5-HT concentrations in the CNS on autopsy [41]. One mechanism that may explain the differences between the central and peripheral serotonergic system in autistic children, is that the latter may have an autoimmune dysfunction affecting brain serotonin receptors. Thus, 7 out of 13 autistic children, but no control subjects, had CSF antibodies directed against brain 5-HT receptors [42]. Another possible mechanism which could determine the serotonergic metabolism in autism is the omega-3 fatty acid status. Indeed, the expression of serotonergic receptors and the turnover of 5-HT are largely modulated by the omega-3 fatty acid status [43]. Previously, we found that autism is accompanied by specific increases in omega-3 fatty acids [44].

In the present study we have controlled for or otherwise may dismiss the intervening effects of variables such as age (all subjects were between 12 and 19 years old), sex (all subjects were males), race (all Caucasians), I.Q. (no subjects with mental retardation were included), drug state of the patients (all subjects were drug free for at least one month), seasonal effects (all tests were carried out in summer 2000), or autistic symptomatology (all patients were without behavior corresponding to the irritability subscale-behavior, e.g. aggression, self-injurious behavior, temper tantrums, irritability, screaming, extreme mood changes). Moreover, we minimized the analytical variability in the biological variables since all assays were run at the same time using the same chemicals. Given the fact that all but one of the autistic subjects of our study group belong to the high- functioning group we cannot exclude wether the findings are specific for the entire autistic population or might be confined to the higher 30% of the overall population of autistic subjects. It remains unclear if or to what degree disturbances in the serotonergic system may contribute to the overall phenotype of autism or only to the degree of severity of autism and /or the presence and severity of associated features. Also from a genetic point of view recent evidence shows that the disorder is genetically heterogeneous. Higher functioning individuals with autism may have another genetic background than lower functioning ones.

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