The platelet-poor plasma 5-HT response to carbohydrate rich meal administration in adult autistic patients compared with normal controls

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INTRODUCTION

Autistic disorder is a childhood-onset disorder, characterized by marked and sustained impairment in social skills, restrained or stereotyped patterns of behaviours or interests and communication deviance. Since a complete understanding of the pathogenesis of autism is lacking, and efficient therapy has yet to be developed, most of the children and adults with autism continue to suffer from the above described symptoms (Wing, 1989). Many studies have indicated the involvement of the 5-HT system in autistic disorder. Most studies examining 5-HT function have focused on whole blood 5-HT content. The carbohydrate-rich meal test (CRMT) is a dietary manipulation that could significantly influence platelet-poor plasma (PPP) 5-HT levels and reflect the responsiveness of the serotonergic system in ‘free’ plasma. In this study, CRMT was used as an indicator of 5-HT responsivity in drug-free adults with autistic disorder (n = 7), compared with normal controls (n = 10). The PPP 5-HT levels were measured at baseline and during 3 h after administration of the CRMT. A significant elevation in PPP 5-HT levels in adult autistic patients was reached 60 min after meal administration (p < 0.03 vs control and p = 0.05 vs baseline) and a significant decrease was noted after 120 min (p < 0.01 vs baseline). In contrast to the biphasic response of the autistic patients, normal controls exhibited a gradual linear increase of PPP 5-HT levels. Our results indicate that in adult autistic patients, the pattern of PPP 5-HT responsivity to a dietary challenge of CRMT is dysregulated compared with normal controls and provide further support for the role of 5-HT in autism. Copyright © 2003 John Wiley & Sons, Ltd.

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Tryptophan competes with large neutral amino acids (LNAA) for penetration of the blood–brain barrier. Intake of a carbohydrate-rich meal increases insulin levels, which, in turn, enhances penetration of most LNAA into peripheral cells (Yokogoshi and Wurtman, 1986), thereby providing Trp a quantitative advantage in competing for carriers of the BBB and entering the brain. This may result in enhanced brain 5-HT synthesis (Blum et al., 1992).

In this study, CRMT was used as an indicator of 5-HT activity in adults with autistic disorder. The PPP 5-HT levels were measured at baseline ($T_0$ time) and for 3 h after administration of CRMT in adults with autistic disorder compared with normal controls. Our preference was to measure PPP 5-HT levels, and not whole blood 5-HT levels since 99% of circulatory 5-HT is contained in platelets (Cook and Leventhal, 1996) and platelet 5-HT content is at least 5-fold higher (up to 170 ng/ml) than the PPP 5-HT level (0.5–33 ng/ml). Thus platelet 5-HT would have significantly contributed to whole blood 5-HT measurements, the levels of which do not represent the actual contribution of the CRMT induced increase in plasma 5-HT level. Moreover fine changes or patterns of change in serotonin levels would not be recognized due to the high platelet serotonin content.

PATIENTS AND METHODS

Study sample

The study group included seven adult individuals with autistic disorder (three women and four men), aged 25.4 ± 4.8 years (mean ± SD), none of whom were hospitalized at least a year before the study. All were recruited from a single centre for adult autistic patients. Participants were drug-free for at least 1 year prior to study commencement.

The diagnosis of autistic disorder was based on DSM-IV criteria. A consensus between two senior board certified child and adolescent psychiatrists was required in order to establish diagnosis. For participation in the study, a minimum score of 30 on the child autistic rating scale (CARS) (Mesibov et al., 1989) was required. The control group included ten healthy volunteers (five women and five men), aged 30.3 ± 4.6 years recruited from the medical staff at the same clinical centre. The exclusion criteria for both groups included chronic or acute physical diseases (e.g. diabetes mellitus), history of alcohol/drug abuse and abnormalities in routine laboratory tests, including abnormal fasting serum glucose levels. The study was approved by the Ness-Ziona Mental Health Center Institutional Review Board. All the participants and their families provided informed consent after having received comprehensive information regarding the nature of the study.

Study procedure

The subjects were administered a carbohydrate rich meal-test after a 14 h fast at a set time (0800 a.m.). In this manner possible changes induced by circadian variation or by previous meals were minimized. The meal-test consisted of two slices of bread (30 g each) topped with 15 g of jam and was composed of 86% carbohydrates, 12% protein and <0.8% fat (a total of 200 Kcal). In each case, venous blood samples were drawn at baseline ($T_0$ time), immediately before the meal, and 60, 120 and 180 min after the consumption of the meal-test. All subjects were instructed to avoid unusual physical activity for 24 h prior to blood collection. During this period the subjects were maintained on a monoamine-free diet. Thus foods containing monoamines (e.g. bananas, Vicia Faba, cheese, etc.) were excluded. Prior to this diet, all subjects had maintained a regular diet.

Laboratory methods

Materials. Serotonin (5-HT) was purchased from Sigma (St Louis, MO). All buffers and solutions were passed through a 0.2 μm filter before injection.

Procedure. Blood samples were drawn into test tubes containing sodium metasulphite and sodium EDTA, and immediately placed on ice until centrifugation. Platelet-poor plasma (PPP) was separated by centrifugation of the blood samples at 1500g for 15 min at 4°C. The resulting PPP was collected and stored at −20°C until assayed. The time between blood collection and plasma preparation (centrifugation and freezing), was similar for both patients and controls and was 3–4 h.

Analytical methods. 5-HT: PPP 5-HT concentrations were determined by an HPLC-ECD method after protein precipitation with 50% TCA containing sodium metabisulphite and sodium EDTA (Tagari et al., 1984). The supernatant was injected into the HPLC system after filtration. The HPLC system consisted of a LDC/Milton-Roy (Riviera Beach, FL) high pressure pump rheodyne injector, a reverse-phase (RP) chromatographic column (BAS, West Lafayette, IN; Octadecyl Silica [ODS] column, 4 × 250 mm,
5 mm) and an electrochemical detector (LC-4c; Bioanalytical Systems [BAS]) equipped with a glossy carbon electrode. The applied potential was +0.64 V. The mobile phase consisted of a monochloroacetate buffer (0.15 M, pH 3.0) containing 2 mM EDTA. For 5-HT determination, methanol was added to yield a final concentration of 5%. All samples were assayed in the same run to avoid inter-assay variability. The lower detection limit was 0.2 ng/ml and the coefficient of variation was 5%.

**Statistical analysis**

The change in PPP 5-HT levels at 60 min, 120 min and 180 min, calculated as a percent of baseline level, was the principal measure of the PPP 5-HT response to CRMT. Two-tailed paired and unpaired Student’s t-test and Wilcoxon signed rank test were used for within- and between-group comparisons, as appropriate. All results are expressed as mean ± SD.

**RESULTS**

No statistically significant difference was found between the two groups in respect to gender (p = 0.4), and a tendency toward younger age was noted in the autistic group (p = 0.053). A significant difference was noted in basal plasma 5-HT levels between the autistic patients and healthy controls (2.5 ± 1.5 ng/ml versus 5.7 ± 2.0 ng/ml; t = 3.5, df = 15, p = 0.004).

The increase in PPP 5-HT levels (by percent of baseline) after administration of the carbohydrate-rich meal in the two groups is depicted in Figure 1. A significant elevation in PPP 5-HT levels in adult autistic patients was observed 60 min after meal administration (234% ± 169%; paired t = 2.06, df = 6, p < 0.03 vs controls [100% ± 80%] and p = 0.05 vs baseline, with Wilcoxon signed rank test), with a decrease to 65% ± 29% at 120 min (paired t = 2.11, df = 6, p < 0.01 vs baseline). At 180 min, the response increased again to 166% ± 167% of baseline (p = 0.4 vs baseline and p = 0.35 vs controls, with Wilcoxon signed rank test).

The response in the healthy control group was linear in contrast to the biphasic response in the adult autistic subgroup. The response of the control group after 60 min, 120 min and 180 min after meal administration was 100% ± 80%, 110% ± 63% and 124% ± 65%, respectively (NS vs baseline). The mean individual PPP 5-HT peak values were significantly higher in the adult autistic group than the normal controls (274% ± 144% vs 154% ± 53%, two-tailed unpaired t = 2.09, df = 15, p < 0.03).

**DISCUSSION**

Our results indicate a biphasic CRMT-induced PPP 5-HT response in adults suffering from autism, compared with a linear pattern in normal control adult subjects. Namely, while in the control subjects, the 5-HT PPP levels increased in a linear fashion, autistic subjects demonstrated a different response pattern with a significant elevation in the first hour, followed by a decline in the second hour and a return to basal range in the third hour (Figure 1).

Our study is consistent with previous challenge tests, which have demonstrated an alteration in the 5-HT system responsivity in autism. McBride et al. (1989) investigated 5-HT system functioning in response to fenfluramine challenge in young male autistic adults. Their findings suggest a reduced 5-HT responsivity in several measures: autistic subjects had a substantially blunted prolactin release (mediated by fenfluramine, a 5-HT releaser 5-HT induced hypothalamic activation), a reduced serotonin-amplified platelet aggregation (mediated by 5-HT2 receptor complex) and a reduced mean number of platelet 5-HT2 receptors. This 5-HT hyper-responsivity as observed is consistent with blunted prolactin release following administration of the 5-HT precursor L-5-hydroxytryptophan as reported by Hoshino et al. (1984). Recently, McDougle et al. (1996a) demonstrated that short-term Trp depletion may lead to a significant worsening of behavioural symptoms in 65% of drug-free adult autistic patients, compared with sham depletion. Patients who showed an exacerbation of symptoms after Trp depletion also had
significant higher predepletion plasma total Trp levels than those autistic subjects who showed no change in symptoms. Thus one may assume that the findings of McDougle et al. (1996a) as well as of ours (an enhanced increase in 5-HT PPP levels in response to CRMT) may implicate a 5-HT dysregulation (potential increased Trp precursor requirement for 5-HT synthesis) in autism. This hypothesis may be further supported by the finding of D’Eufemia et al. (1995) who has demonstrated a low serum Trp/LNAA ratio in idiopathic infantile autism. This lowered ratio was mainly due to the considerable increase of LNAA.

The above studies are part of the growing evidence for the importance of the 5-HT system function in autism. The first indication for the involvement of the 5-HT system in autism was reported four decades ago, by Scharff and Freedman (1961) who demonstrated high whole blood 5-HT levels in autistic children. Today, it is estimated that 25%–30% of autistic patients exhibit high whole blood 5-HT levels (hyper-serotonaemia). However, it remains unclear whether this phenomenon reflects a peripheral compensatory mechanism of serotoninergic regulation or a primary defect (Anderson et al., 1990). Interestingly, at least one study has failed to demonstrate any difference in PPP 5-HT levels between autistic children and their first-degree relatives (Cook et al., 1988). In the CSF, however, levels of 5-hydroxyindolacetic acid, the 5-HT primary metabolite were similar (Narayan et al., 1993) or reduced (Cohen et al., 1974) compared with matched control subjects. Another strong indication for the involvement of 5-HT in autism arose from studying the clinical efficacy of 5-HT modulating agents in autism. Indeed, clomipramine (a non-selective 5-HT reuptake inhibitor) (McDougle et al., 1992) and fluvoxamine (a selective serotonin reuptake inhibitor) (McDougle et al., 1996b) have both been demonstrated to improve some symptomatology in adult autistic patients.

Thus our study is consistent with the above studies in showing a dysregulation of the 5-HT system in adults suffering from autism, compared with control healthy subjects. This, in turn, may suggest an alteration in the equilibrium between the platelet 5-HT content and the PPP 5-HT content (e.g. platelet 5-HT uptake and efflux). Until now, no consensus has been reached concerning the alterations in 5-HT platelet uptake, storage and efflux in autistic subjects compared with normal controls (for review see Anderson et al., 1990). Interestingly, as can be noted, autistic patients had significantly lower levels of 5-HT at baseline. While this is, in and of itself, an interesting observation and requires more investigation, in this study we were more interested in demonstrating the pattern and change in 5-HT levels following a carbohydrate rich food challenge. It should also be remembered that while the overall diet as such does not change basal serotonin PPP levels, nevertheless significant short-term changes do often occur after consumption of a meal rich in either carbohydrate or protein (Blum et al., 1992) as further observed in this study.

Limitations of our study include the small sample size, partly due to difficulties in recruiting drug free (>1 year) patients as well as the difference (although not statistically significant) between the ages of the groups. This drawback may be partially overcome by the stability of 5-HT platelet levels after the age of 9 years (Ritvo et al., 1971). Furthermore, while the possibility exists that the contribution of the plasma pool may be altered by experimental factors such as platelet aggregation during blood extraction, our handling of the samples would ensure that no platelet serotonin enters the samples. It is most important to note that levels of serum serotonin (representing the sum of platelet and plasma serotonin) are normally found in the range 120–150 ng/ml (e.g. Pietraszek et al., 1992). In our investigation, levels of PPP serotonin were found much lower, in the range 1–7 ng/ml, thus further supporting the suggestion that limited contamination occurred, if any, of the PPP by platelets.

In conclusion, our results may indicate that the 5-HT system, at least at the peripheral level, is dysregulated in autistic disorder. This dysregulation may be genetically determined, as has been demonstrated for at least for one component of the 5-HT system, namely the 5-HT transporter (Tordjman et al., 2001), and thus may have therapeutic implications including the use of 5-HT modulating agents (McDougle et al., 1996b). Furthermore, since Trp depletion may lead to aggravation of some autistic symptoms (McDougle et al., 1996a), it may be hypothesized that enhancement of Trp availability (e.g. Trp supplement or carbohydrate rich diet) may lead to relative amelioration of autistic symptoms. In the future, efforts should be exercised in characterizing various subgroups of autistic patients in relation to 5-HT function. Further studies are clearly warranted in order to definitively clarify the involvement of 5-HT in autism as well as response to 5-HT modulating agents.

REFERENCES


