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Copper in developmental stuttering:
a study of plasma copper, ceruloplasmin, and estimated free copper

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Abstract

It has previously been reported that men with developmental stuttering showed reduced concentration of copper in the blood, and a negative correlation between the copper level and the severity of stuttering. Disorders of copper metabolism may result in dysfunction of the basal ganglia system and dystonia, a motor disorder sharing some traits of stuttering. It has been shown that copper ions affect the dopamine and the GABA systems. With this background we investigated the plasma level of copper, the copper binding protein ceruloplasmin, and the estimated level of free copper in stuttering adults. Sixteen men with developmental stuttering were compared with 16 men without speech problems. The samples were assayed in one batch in a pseudo-random and counterbalanced order. No significant differences were found between stuttering men and the control group in any of the biological variables, and no relation between copper and the severity of stuttering was shown. This result indicates that there is no relation between developmental stuttering and copper in the main population of stuttering adults.

Keywords: Stuttering, copper, ceruloplasmin

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1. INTRODUCTION

Stuttering is a frequent speech motor disorder of unclear etiology. A relation to basal ganglia dysfunction has been suggested [1–3]. The existence of aberrations of the dopamine system is supported by an FDOPA-PET study indicating increased dopamine synthesis in stuttering adults [4], and by improvement of some cases of stuttering with pharmacological treatment using D2 receptor antagonists [5].

There are several parallels between stuttering and *task specific dystonia* like writer's cramp [2,3,6]: excessive muscular contraction limited to specific automated sequential movements (for example writing or playing an instrument), blocking or altering the sensory feedback often improves the condition, some cases are responsive to drugs affecting the dopamine system, and lesions causing dystonia or stuttering are often located in the lentiform nucleus.

A possible relation between copper (Cu) and developmental stuttering is suggested by a study by Pesak and Opavsky [7,8]. A group of 16 male persons with developmental stuttering had significantly lower serum Cu levels than controls ($p < 0.001$): 14.1 µmol/l serum Cu (SD 2.1 µmol/l) in the stuttering group and 17.2 in the control group (SD 2.3 µmol/l) [8], making a difference in means of 3.1 µmol/l. Furthermore, the level of Cu showed a negative correlation ($r = -0.57$) with a measurement of the severity of stuttering (uncertain phonation starts). This report of low serum level of Cu in stuttering is interesting in relation to the suggestions of stuttering as a basal ganglia disorder with traits of dystonia. It is well known that disorders of Cu metabolism can result in basal ganglia dysfunction. In Wilson's disease the binding of Cu to the protein ceruloplasmin (Cp) is impaired, resulting in deposition of Cu in the liver and in the basal ganglia while the blood level of Cu and Cp is lower than normal. The neurological symptoms of Wilson's disease are predominantly movement related, like slurred speech, dystonia, and tremor [9]. A relation between Cu and dystonia has also been suggested in patients without Wilson's disease [10].

Cu ions have been shown to have a potent inhibitory effect on GABA(A) receptors [11], and to have pronounced effects on the dopamine system [12]. GABA and dopamine are of critical importance for the functions of the basal ganglia [2]. Thus, anomalies in the exact regulation of Cu might theoretically be related to different types of behavioral disorders, for example stuttering. Excess tissue Cu has been suggested to be involved in schizophrenia. Bowman and Lewis [13] concluded that the hypothesis of a relation between Cu and schizophrenia has neither been compellingly demonstrated nor convincingly refuted.

Normally about 95% of the total Cu in serum is bound to Cp. Each Cp molecule binds six to eight Cu atoms. Cp not containing Cu has a much shorter half-life than Cp with Cu. As a result, the blood levels of Cu and Cp normally show high correlation. Estrogens increase the level of serum Cp and Cu, and these levels are normally higher in women than in men, with larger difference when using estrogenic contraceptives or during pregnancy. When binding of Cu to Cp is impaired, as in Wilson's disease, the amount of "free" Cu in the blood is increased, while the total blood Cu is reduced [14]. The level of free Cu can be estimated by comparing the

levels of total serum (or plasma) Cu and Cp. The amount of free Cu ions in the blood might be an interesting factor when discussing possible effects of Cu on neural systems.

Are the levels of Cu and Cp influenced by stress? Cu is reported to be increased by physical stress, like inflammation and infections [15]. A test of 24 hours intermittent speed driving resulted in 37% increase of Cp [16]. However, the effect of emotional stress does not seem clear. It might be theoretically possible that emotional and physical stress caused by stuttering could increase the level of Cp and Cu, but there are no indications of such effect.

The result from Pesak and Opavsky [8] of low serum Cu in stuttering men was supported by a pilot study of stuttering men in our laboratory, without control group (n = 23, plasma Cu = 14 μmol/L to be compared with 14.1 μmol/L Cu in the study by Pesak and Opavsky [8]). The pilot study also indicated a low level of ceruloplasmin and a high level of estimated free copper. Therefore we conducted the controlled study reported in this paper.

The general purpose of the present study was to investigate possible differences regarding Cu and Cu-related measures in persons who stutter. The primary purpose was to replicate the study by Pesak and Opavsky [8] of Cu in men with stuttering, and to expand it by estimation of the level of free Cu based on analysis of Cp. In the present study also females were included, but the statistical analysis was limited to men, because of difficulties in achieving a sufficient number of female participants who stutter, especially since the level of Cu is affected by estrogenic contraceptives.

2. METHOD

2.1. Estimation of Stuttering Severity

The severity of stuttering was estimated as a global severity score based on rating of video recordings of speech samples, ranging from 0 (no stuttering) to 7 (very severe). The global rating was determined by first estimating scores for superfluous muscular activity and scores for the proportion of speech time with symptoms of stuttering, during spontaneous speech and during reading aloud. Thereafter the global score was set as an approximate composite of the sub-scores. Scoring was done independently by two raters, with rater agreement indicated by Pearson correlation coefficient r = 0.92 and rater bias = 0.84 (i.e., the difference of the mean scores of the raters). The mean of the two ratings was used for the final analysis. In 5 of the 20 cases the video recordings were judged to be clearly not representative of the stuttering in these cases, for example because of situational variations or use of fluency techniques. To increase to validity of the severity ratings the global scores for these 5 cases were adjusted based on additional information (from observations and interviews).

2.2. Participants

The participants were 16 males (age 22–48, mean 39.2) and 4 females (age 19–43, mean 34.2) with developmental stuttering, and 16 males (age 25–59, mean 37.6) and 12 females (age 24–50, mean 37.7) without speech problems. Persons with active inflammatory disease, diabetes, liver disorder, estrogenic drugs, corticosteroids, or diuretic medication were excluded, as well as control persons with a history of stuttering or cluttering in the family, or with neurological or psychiatric disorders. The mean stuttering severity score for males was 3.2 (range 0.5–7) and for females 4.1 (range 1.5–5.5). The study was performed as part of a larger study of stuttering, approved by the Lund University Research Ethics Committee.

2.3. Biochemical Methods

Blood samples were collected between 12:20 and 4:00 p.m., centrifuged, and stored at –70°C. The analyses were performed in one batch, with stuttering persons and controls intermingled in a pseudo-random and counterbalanced order. Plasma Cu was assayed using an atomic absorption spectrophotometer (Perkin-Elmer 1100B). Plasma Cp was analyzed by immunologic method (Hitachi 917) using rabbit anti-human ceruloplasmin antibodies (Dakopatts, Denmark, Cat. No. A031) and the calibrator BCR Reference Material CRM 470 (Dakopatts). An estimation of the concentration of "free" Cu unbound to Cp was calculated with the assumption that 1 mg Cp binds 3.2 µg Cu [17], resulting in the formula: estimated free Cu = Cu – Cp * 50.4.

2.4. Statistics

Group differences relating to Cu and Cp levels for men were evaluated by independent samples t tests, with the software Statistica 6.0 (StatSoft, Inc). Alpha was set at 0.05 for all statistical tests. One-tailed tests were used for plasma Cu and Cp, based on the result of Pesak and Opavsky [8]. Two-tailed test was used for estimated free Cu. Statistical power was calculated using PS software version 2.1.31 [18].

3. RESULTS

No differences were found between stuttering men and the control group in any of the biological variables. No relation between the levels of plasma Cu, or estimated free Cu, and the severity of stuttering was found. Also the group of women (N = 4) with stuttering showed results similar to the controls. The correlation between plasma levels of Cu and Cp was r = 0.95 (based on all available cases, including females using estrogenic contraceptives). Table 1 summarizes the means and standard deviations of the participants.

Independent samples t test showed no statistical difference in the level of plasma Cu between stuttering men and controls ($t(30) = 0.16$, $p = 0.56$, one-tailed).

Likewise, t tests of levels of Cp (t(30) = 1.10, p = 0.86, one-tailed) and estimated free Cu (t(30) = 0.78, p = 0.44, two-tailed) showed no difference between stuttering men and controls. The level of plasma Cu or estimated free Cu showed no significant correlation with measures of stuttering severity. The non-significant tendency was towards a positive correlation, with r = 0.33 between plasma Cu and the severity score.

Table 1. Means and standard deviations for plasma Cu, plasma ceruloplasmin, and estimated level of free Cu in plasma, in participants with and without stuttering.

		P-Cu (µmol/L)			P-Cp (g/L)		Free Cu (µmol/L)	
Group	*n*	*M*	*SD*	*range*	*M*	*SD*	*M*	*SD*
Males								
Stuttering	16	15.49	1.9	11.4–18.7	0.219	0.030	4.4	1.5
Control	16	15.35	2.9	9.1–20.7	0.207	0.034	4.9	2.0
All	32	15.42	2.4	9.1–20.7	0.213	0.032	4.7	1.8
Reference interval [14]				11.0–22.0	0.18–0.45			
Females								
Stuttering	4	15.25	2.5	13.0–17.8	0.225	0.021	3.9	1.8
Control	12	17.14	2.9	13.0–22.2	0.245	0.043	4.8	1.7
All	16	16.67	2.7	13.0–22.2	0.240	0.039	4.6	1.7
Reference interval [14]				12.6–24.3	0.18–0.45			

4. DISCUSSION

The result of this study points clearly against any relation between developmental stuttering in adults and plasma levels of Cu. Pesak and Opavsky [7] found lower Cu in stuttering men and a negative correlation between Cu and the severity of stuttering but this was not seen in the present study. On the contrary, the mean Cu level of the stuttering group of men was slightly higher than in the controls, and the non-significant correlation between Cu and the severity of stuttering was in the opposite direction than what was reported by Pesak and Opavsky. In the study by Pesak and Opavsky [8] the group of stuttering men showed a 3.1 µmol/L lower level of Cu than the control group. The present study had a power of 0.97 to detect a population difference of this magnitude, or a power of 0.80 to detect a difference in population means of 2.1 µmol/L (one-tailed tests). Jones et al. [19] argued for the importance of accumulation of null results with adequate power in research on

stuttering, in order to rule out the possibility of causal relations. A minimum power of 0.80 was recommended.

For all groups the estimated levels of free Cu were higher than expected, corresponding to about 70% binding of Cu to Cp according to the calculation, to be compared with the expected level of about 95% binding [14]. The level of Cp was lower than expected in all groups, for example about 30% lower than the levels in healthy individuals reported by Varela et al. [20]. It is possible that differences in method have resulted in relatively low levels of Cp in the present study, with increased level of estimated free Cu as a consequence. This does not, however, affect the comparisons between the groups since the levels of Cu and Cp showed a high correlation, r = 0.95, implying good consistency of the measurements.

Also the levels of plasma Cu were generally lower in the present study compared with published studies of healthy individuals. The pooled mean for men in this study was 15.4 μmol/L, to be compared with published mean levels of 18.6 [21], and 17.2 (age 18–60) [22]. The same tendency is shown for females. However, the high levels of estimated free Cu in the present study points against a bias towards low measurements of total plasma Cu. At all events, the mean level of plasma Cu for stuttering men was higher in this study than in the study of Pesak and Opavsky [8], 15.5 vs. 14.1 μmol/L. A possible cause of differing results may be heterogeneity in the stuttering population, but in the present study there is no indication of any subgroup with especially low plasma Cu.

It can be noted that Pesak and Opavsky [8] reported that the Cp levels were within normal limits in the group of stuttering men with reduced level of serum Cu. If these measurements were correct this would point to a *reduced* concentration of free Cu in these stuttering men. The assumed normal concentration of free Cu is about 1 μmol/L, to be compared with the 3.1 μmol/L reduction of mean Cu level in this group of stuttering men. The finding of normal level of Cp raises the question of a possible bias in the analysis of Cu in this stuttering group. In Pesak and Opavsky [7] it is mentioned that the control group was analyzed subsequently, which implies a risk for differences in the analysis of the two groups.

In summary, the results of the present study indicate that there is no relation between developmental stuttering and copper in the main population of stuttering adults.

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