Sympathetic overactivity and plasma leptin levels in Rett syndrome

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Abstract

Rett syndrome (RTT) is a severe developmental–neurological disorder, characterized by profound and progressive loss of intellectual functioning, occurring after a period (of at least 6 months) of normal development with classic stereotype hand movements, gait ataxia, jerky truncal ataxia, deceleration of brain and body organ growth and cardiac dysautonomia. Pathogenesis of sympathetic overactivity in RTT is unknown, but a previous study observed increased plasma leptin levels in Rett girls and it is well known the role of leptin in the regulation of sympathetic nervous system activity. Aim of our study is to evaluate a relationship between plasma leptin levels and sympathetic activity in RTT. Thirty-two female patients (12.1 ± 6.3 years), affected by RTT were enrolled in the study. In all the subjects, we analyzed heart rate variability, QT corrected interval and plasma leptin levels. A significant correlation was found between plasma leptin levels and LF/HF (expression of sympatho-vagal balance) (Spearman \( r = 0.44, p = 0.001 \)). There is also a significant negative correlation between HF component (expression of vagal activity) and plasma leptin levels (Spearman \( r = -0.037, p = 0.03 \)) and a positive correlation between LF component and plasma leptin levels (Spearman \( r = 0.047, p = 0.01 \)). These results show that in RTT higher plasma leptin levels appear to be associated with sympathetic overactivity, suggesting a role for leptin in cardiac dysautonomia.

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Rett syndrome (RTT) is a severe developmental–neurological disorder [19], affecting approximately 1 in 10,000–15,000 females [12]. It is characterized by apparently normal development for the first 6–18 months of life, followed by profound and progressive loss of intellectual functioning, with classic stereotype hand movements, gait ataxia, jerky truncal ataxia, deceleration of brain and body organ growth with many autistic features. There is a wide variability in the rate of progression and severity of the disease and as well as the classical form of RTT, there are a number of recognized atypical variants [11]. In almost 80% of these cases, there is a mutation in the X-linked gene methyl-CpG-binding protein 2 (MECP2); more recently, mutations in two other genes, cyclin-dependent kinase like 5 (CDKL5) [14] and Netrin G1 [5] have been identified in patients with a clinical phenotype that strongly overlaps with RTT.

In RTT, the autonomic nervous system is abnormal at various levels, from the central to the peripheral nervous system; our previous studies [9,8] showed that girls with classic RTT had significantly lower heart rate variability, associated with an increase of adrenergic tone, in comparison with age-matched healthy girls. Reduction of heart rate variability progresses with age and with the clinical stage of the syndrome; however, the pathogenesis of cardiac dysautonomia in these girls is still unknown.

It is well known the role of leptin in the elevation of sympathetic nervous system activity: leptin plays a pivotal role in a wide variety of organ systems including the reproductive, renal and cardiovascular systems [17]; in addition to its direct effects on energy metabolism and caloric intake, leptin exerts several circulatory effects that appear to be mediated by an interaction with the sympathetic nervous system and with the major reflexogenic area involved in cardiovascular homeostatic control [7]. Previous data support the concept that sympathoactivation to leptin is due to the action of this hormone in the central nervous system: in fact direct administration of leptin to the third cerebral ventricle increases sympathetic nerve activity [10] and furthermore sympathoactivation to intravenous leptin can be...
abolished completely by selective lesioning of the hypothalamic arcuate nucleus [10] suggesting that cardiovascular effects of leptin might be evoked by the action of this hormone in the arcuate nucleus of the hypothalamus [16].

Our previous study [4] showed that, in patients with classic RTT and preserved-speech variant, leptin values are significantly higher than controls: in all patients the increased leptin concentrations are not associated to obesity, suggesting that leptin might participate to clinical manifestations other than weight balance.

In this view, aim of our study is to evaluate a link between plasma leptin levels and sympathetic activity in Rett girls.

Thirty-two female patients, age range 3–31 years (12.1 ± 6.3 years), affected by RTT were enrolled in the study. Patients were treated with anticonvulsant drugs (carbamazepine and valproate) and they were followed in the Department of Child Neurology and Psychiatry of the University Hospital of Siena, Italy. Diagnosis of RTT was based on clinical criteria and molecular analysis of the MECP2 gene. Blood samples (3 mL) were drawn from an antecubital vein in the supine position, in the morning after an overnight fast. The study was approved by the Ethics Committee of the University Hospital of Siena, Italy. The parents of all the children provided written informed consent.

Plasma leptin levels were determined by the enzyme-linked immunosorbent assay method using ready kits of Quantikine® Human Leptin Immunoassay (R&D Systems, Lille, France). Sensitivity of undiluted samples was 7.8 pg/mL. Inter- and intra-assay coefficients of variation were 5.4% and 3.3%, respectively.

In each girl, a 12-lead electrocardiogram was continuously monitored and recorded for up to 10 min, in supine position, during spontaneous breathing. Commercially available imaging system (Cardioline ECT WS 2000, Remco Italia, Vignate-Milano, Italy) was used. QRS detection and RR interval measurement were automatically performed, looking for the R wave peak as a reference point. Premature beats, missed beats and artifacts were visually identified using an interactive graphic interface and corrected by the operator. In this way, an RR tachogram was obtained, that is, a discrete series of successive RR intervals as a function of the number of recognized QRS complexes. Algorithm, used for the analysis of the tachogram, was a spectral method (fast Fourier transformation). Three main spectral components were distinguished in a spectrum calculated from short-term recordings of 5 min:

- a very low frequency (VLF) component: <0.04 Hz;
- a low frequency (LF) component: range 0.04–0.15 Hz
- a high frequency (HF) component: range 0.15–0.4 Hz.

The measurement of VLF, LF and HF power components and of total power was made in absolute values of power (ms²). LF and HF components were also measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component (the representation of LF and HF in normalized units emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system and tends to minimize the effect of the changes in total power on the values of LF and HF components). The ratio of low to high frequency (LF/HF) was calculated as an expression of the sympathovagal balance [13].

The QT interval, determined by the longest hand-measured QT interval in any lead, was corrected for the heart rate by the Bazett method to yield the QTc value: QTc was calculated by dividing the QT interval by the square root of the R–R interval, excluding those intervals shorter than 521 ms and longer than 1111 ms, because Bazett’s formula considers values exceeding this range as unreliable [6]. The QTc was considered abnormal if greater than 0.44 s.

All results are presented as means ± S.D. To determine if there was a correlation between plasma leptin levels and each heart rate variability parameters (total power, VLF, LF, HF, LF/HF) and QTc interval, a nonparametric correlation Spearman analysis was performed using Graphpad Instat (version 3.06 for Windows) computer software. A p-value below 0.05 was considered statistically significant. The nonparametric Mann–Whitney test for unpaired data was performed to compare leptin plasma levels in Rett children with QTc prolongation versus Rett children with normal QTc.

Descriptive statistics for the data are presented in Table 1. According to previous studies [9,8], in Rett children we found a reduction of heart rate variability and a sympathetic imbalance (expressed by an increased LF/HF ratio); the QTc interval was prolonged in 59% of Rett girls (19/32 patients).

A significant correlation was found between LF/HF (expression of sympatho-vagal balance) and plasma leptin levels (Spearman $r = -0.56, P = 0.0008$) and in normalized units (Spearman $r = -0.037, P = 0.03$) (Fig. 2c and d).

No correlation was found between plasma leptin levels and total power, LF spectral component measured in absolute value of power (Fig. 2a), QTc interval. However, there was also a significant positive correlation between plasma leptin levels and LF component measured in normalized units (Spearman $r = 0.047, P = 0.01$) (Fig. 2b).

### Table 1

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<th>Age, heart rate variability parameters, RR, plasma leptin levels, QTc interval and blood pressure values in Rett children ($n = 32$)</th>
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Values are expressed as mean ± standard deviation.
Fig. 1. Correlation between plasma leptin levels and sympatho-vagal balance (LF/HF) (Spearman \( r = 0.44, P = 0.001 \)).

Furthermore, Rett girls with QTc prolonged \((n = 19, \text{QTc} > 0.44 \text{ s})\) in comparison with Rett patients with normal QTc \((n = 13, \text{QTc} < 0.44 \text{ s})\) showed no significant differences for plasma leptin levels \((37.9 \pm 26.7 \text{ ng/ml} \text{ vs. } 18.4 \pm 14.8 \text{ ng/ml}; p = 0.6)\).

The evaluation of heart rate variability confirmed the presence of a cardiac dysautonomia in Rett girls, as observed in previous studies \([9,8]\): in fact, high values of LF/HF ratio suggest a sympathetic imbalance. Furthermore, our results showed a correlation between plasma leptin levels and LF/HF suggesting a role for leptin in the genesis of cardiac dysautonomia of Rett girls. In particular, plasma leptin levels correlate positively with LF (measured in normalized units) and negatively with HF (measured in normalized units and in absolute value of power).

It is well known that HF power seems to reflect vagal tone and LF may have contributions from both vagal and sympathetic; but the sympathovagal balance hypothesis suggests that activation of sympathetic versus parasympathetic efferent neurons normally is accompanied by inhibition of the other efferent limb and finally LF/HF values suggest a sympathetic imbalance in subjects with high leptin values and a vagal predominance in Rett children with low leptin plasma levels.

Pathogenesis of sympathetic overactivity in RTT is unknown; in our previous papers \([1,2]\) we hypothesized that an altered neurotrophin signalling, a serotoninergic dysfunction, and a deficiency of substance P may contribute to an impairment of the autonomic nervous system, resulting in cardiac dysautonomia.

In Rett girls, the evidence of increased plasma leptin levels \([4]\) suggested a link with cardiac autonomic alterations. Leptin has structural homology to tumor necrosis factor alpha (TNF-\(\alpha\)), interleukin 6 (IL-6), leukemia inhibitory factor, granulocyte-colony stimulating factor, glycoprotein 130 (gp130) and other cytokine family proteins, and is therefore considered a cytokine-like substance \([3]\). Previous studies have shown that plasma leptin may affect autonomic nervous system activity: the results concerning the effect of plasma leptin on the autonomic nervous system at the cardiovascular level are controversial, but recently Paolisso et al. \([15]\) observed that the increase of plasma leptin concentrations is associated with an increase in the LF/HF ratio, an index of cardiac sympathovagal balance, independently of anthropometric characteristics, demonstrating that the association between the variation in plasma leptin concentration and the cardiac autonomic nervous system activity was independent of body fat.

Fig. 2. (a) Correlation between plasma leptin levels and LF measured in absolute values (Spearman \( r = -0.26; p = 0.14, \text{not significant} \)). (b) Correlation between plasma leptin levels and LF measured in normalized units (Spearman \( r = 0.047, p = 0.01 \)). (c) Correlation between plasma leptin levels and HF measured in absolute values (Spearman \( r = -0.56, P = 0.0008 \)). (d) Correlation between plasma leptin levels and HF measured in normalized units (Spearman \( r = -0.037, p = 0.03 \)).
Leptin binding sites have been found in brain regions that are important in cardiovascular control: the intracerebroventricular or intravenous administration of leptin produces marked changes in arterial blood pressure, heart rate, sympathetic nerve activity and renal excretory function in rats and rabbits [18]. On the other hand, plasma leptin levels were reduced in patients with idiopathic sympathetic nerve degeneration and low sympathetic tone. In RTT, it is possible that alteration of various synergic factors determines sympathetic overactivity and, in this view, leptin could represent one of these factors.

The results of our study may contribute to understand the pathogenesis of cardiac dysautonomia in Rett girls: in fact leptin may play a role in the sympathetic overactivity observed in RTT.

References