Pediatr Cardiol 25:394–396, 2004
DOI: 10.1007/s00246-002-0406-y

Pediatric Cardiology

Nerve Growth Factor Plasma Levels and Ventricular Repolarization in Rett Syndrome

F. Guideri,[1] M. Acampa,[1] G. Calamandrei,[2] L. Aloe,[2] M. Zappella,[3] Y. Hayek[3]

[1]Department of Clinical Medicine and Immunological Sciences, University of Siena, Siena, Italy

[2]Institute of Neurobiology, CNR, Rome, Italy

[3]Department of Child Neurology and Psychiatry, University of Siena, Siena, Italy

Abstract. Rett syndrome is a severe neurological developmental disorder. In this syndrome, the high incidence of sudden death is correlated with an alteration of ventricular repolarization. The purpose of this study was to evaluate plasmatic levels of nerve growth factor (NGF) in Rett patients with prolonged corrected QT (QTc) interval in comparison with those of Rett patients with normal QTc. We observed 23 female Rett patients (9.9 ± 4.7 years). NGF plasma levels and QTc interval were measured in all patients. Student *t*-test was performed for statistical analysis. NGF plasma levels were significantly lower in Rett patients with QTc interval prolongation (QTc > 0.44 sec) in comparison with Rett patients with a normal QTc interval (4.5 ± 4.5 vs 11 ± 8.3 pg/ml, $p = 0.02$). The alteration of NGF levels, observed in Rett patients with a long QTc interval, may explain the presence of an altered ventricular repolarization associated with a higher risk of cardiac arrhythmias.

Keywords: Sudden death — Rett syndrome — Corrected QT interval — Nerve growth factor

Rett syndrome is a severe neurological disorder, occurring almost exclusively in females, characterized by onset in childhood, psychomotor regression after at least 6 months of apparently normal life, classic stereotype hand movements (hand washing/wringing or clapping/tapping hand to mouth), gait ataxia, jerky truncal ataxia, head circumference stagnation, loss of purposeful hand movements, and breathing rithms alteration [3]. There is usually a deceleration of brain and body organ growth with many autistic features. The incidence of Rett syndrome is 1/10,000–15,000 [14]. Recent studies have shown in almost 80% of cases of classic Rett syndrome the presence of a methyl-CpG-binding protein 2 (MECP2) gene mutation [7].

The mortality rate in Rett syndrome is 1.2% per year; of these deaths, 26% were sudden and unexpected [17]. In comparison, the incidence of sudden, unexpected death in the general population between 1 and 22 years of age was 1.3 per 100,000 patients per year [9].

The pathogenesis of sudden death in Rett syndrome is unknown, but electrical instability is a prime suspect. A prolonged corrected QT (QTc) interval has been reported in Rett syndrome. Sekul et al. [23] reported QTc prolongation (QTc > 0.45 sec) in 41% of their cohort, with an increase in the mean value across clinical stages, and our previous study [13] confirmed these data. Sekul et al postulated that a prolonged QTc interval could be a possible cause of sudden death in Rett syndrome. The prolonged QT syndrome is a serious and potentially lethal cardiac disorder. The condition is believed to be caused by abnormal repolarization after ventricular systole, and it has been attributed to an abnormality at the myocardial cellular level.

Recently, we observed an abnormal developmental profile of serum nerve growth factor (NGF) levels in Rett disorder [4]: whereas NGF levels increased significantly with age in controls, in Rett females we observed a progressive age-dependent decrease in NGF. These data suggest a role for NGF in the pathogenesis of Rett syndrome.

NGF is an important modulator of neuronal plasticity at synapses formed by central nervous system neurons; in the periphery NGF influences neuronal survival and differentiation in the developing sympathetic and sensory nervous systems and enhances neuritic growth and target innervation, indicating that it functions as a modulator of sympathetic synaptic transmission [20].

NGF is expressed in the heart and in other sympathetic targets at approximately the time of in-

Correspondence to: F. Guideri, *email:* guideri@unisi.it

itial innervation [6]. Previous studies showed that treatment of neonatal rats with the antiserum to NGF led to an abnormal heart innervation pattern with an immature electrophysiological response to α-adrenergic stimulation [21]. Furthermore, when cardiac sympathetic innervation in neonatal rats is retarded by antiserum to NGF, there is a corresponding increase in the QT interval on electrocardiogram. The prolonged QT interval may be explained by the retarded pattern of both nexal and desmosomal junction formation and by thedispersal of action potential duration [24].

In this study, we evaluate the plasmatic levels of NGF in Rett children with prolonged QTc interval in comparison to those of Rett children with normal QTc interval.

Patients and Methods

We observed 23 females with Rett syndrome; their diagnosis was confirmed independently by two child neuropsychiatrists (YH and MZ) and clinical stage was determined according to the system of Hagberg and Witt-Engerström [15]. All patients showed mutation of the gene MECP2. NGF plasma levels and QTc interval were measured in all patients. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Blood NGF Levels

Blood for all subjects was drawn in the morning between 8:00 and 10:00 AM. Plasma was collected from heparinized whole blood, supplemented with 20 mg/ml aprotinin, and stored at −86°C until NGF assay. Briefly, polystyrene 96-well microtube immunoplates (Nunc) were coated with affinity-purified, polyclonal goat anti-NGF antibody and diluted in 0.05 *M* carbonate buffer (pH 9.6). Parallel wells were coated with purified goat IgG (Zymed, San Francisco, CA, USA) in order to evaluate the nonspecific signal. Following overnight incubation at room temperature and 2 hours of incubation with a blocking buffer (0.05 *M* carbonate buffer, pH 9.5, 1% BSA), plates were washed three times with 50 m*M* Tris-HCl (pH 7.4), 200 m*M* NaCl, 0.5% gelatin, and 0.1% Triton X-100. After extensive washing of the plates, the samples and the NGF standard solutions were diluted with sample buffer [0.1% Triton X-100, 100 m*M* Tris-HCl (pH 7.2), 400 m*M* NaCl, 4 m*M* EDTA, 0.2 m*M* PMSF, 0.2 m*M* benzethonium chloride, 2 m*M* benzamidine, 40 U/ml aprotinin, 0.05% sodium azide, 2% BSA, and 0.5% gelatin], distributed among the wells, and left at room temperature overnight. The plates were then washed three times and incubated with 4 mU/well anti-β-NGF-galactosidase (Boehringer Mannheim, Germany) for 2 hours at 37°C, and after further washing, 100 µl of substrate solution [4 mg/ml of chlorophenol red (Boehringer Mannheim); substrate buffer: 100 m*M* HEPES, 150 m*M* NaCl, 2 m*M* $MgCl_2$, 0.1% sodium azide, and 1% BSA] was added to each well. After incubation for 2 hours at 37°C, optical density was measured at 575 nm using an enzyme-linked immunosorbent assay reader (Dynatech), and the values of standards and samples were corrected by taking nonspecific binding into consideration. Under these conditions, the sensitivity was 3 pg/ml, the recovery of NGF ranged from 80 to 90%, and cross-reactivity with other related neurotrophins of the NGF family, such as BDNF and NT-3, was less than 3% [2, 26]. Data were represented as pg/ml and all assays were performed in triplicate.

QTC Interval

Twelve-lead ECGs were recorded simultaneously. Standard criteria were applied for the QT measurement [12]. QT intervals were measured from the onset or Q wave or the onset of the QRS to the end of T-wave, defined as return to the T-P baseline. When U waves were present, the QT was measured to the nadir of the curve between the T and U waves.

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 msec and longer than 1111 msec; Bazett's formula considers values exceeding this range to be unreliable [11]). The QTc interval was considered abnormal if greater than 0.44 sec.

Statistical Analysis

NGF plasma levels and age were compared in individuals with Rett syndrome with and without QTc interval prolongation by means of Student *t*-test for unpaired data.

Results

Of the 23 individuals with Rett syndrome, 20 had classic Rett syndrome and 3 were classified as preserved speech variants. Age ranged from 4 to 21 years (9.9 ± 4.7 years).Theree patients were in clinical state II, 18 patients were in clinical stage III, and 2 patients were in clinical statge IV of the disorder. Eighteen patients had a history of seizures, 16 of whom were taking anticonvulsants (5 were taking lamotrigine, 8 were taking carbamazepine, and 3 were taking sodium valporate).

The QTc values of the Rett syndrome cohort ranged from 0.39 to 0.49 sec, with a mean of 0.44 sec. A prolonged QTc (>0.44sec) was identified in 12 patients (57%) (10.9 ± 4.8 years) The corrected QT interval in these patients ranged form 0.45 to 0.49 sec (0.46 ± 0.01 sec).

A total of 18 patients were assigned to stage III Rett syndrome, of whom 10 were found to have a prolonged QTc value. One out of 3 patients in stage II and 1 out of 2 patients in Stage IV had QTc prolongation.

In 11 Rett girls (10.9 ± 4.8 years) with normal QTc interval (<0.44 sec), QTc values ranged from 0.39 to 0.44 sec (0.41 ± 0.01 sec).

There were no significant differences (i.e., for age) between the two groups of Rett girls. NGF plasma levels were significantly lower in Rett children with

QTc interval prolongation in comparison wth Rett girls with normal QTc values (4.5 ± 4.5 vs 11 ± 8.3 pg/ml, $p = 0.02$).

Discussion

A variety of studies published in recent years have investigated the role of NGF and other molecules of the same family [1, 5, 16] in regulating cardiovascular development and modulating the vascular response to nerve injury. For example, it has been reported that NGF protects against postischemic dysfunction of the sympathetic coronary innervation [1], is reduced in human and experimental heart failure [16], is involved in nerve sprouting associated to cardiac arrest [5], and regulates intramyocardial vessel stabilization [8].

A role for neurotropic factors has been postulated in Rett syndrome [19], and complex interactions have been suggested among cortical morphogenesis, neuronal differentiation, and nerve growth factor. NGF expression in the brains of patients with Rett syndrome is reduced [19], and this observation in confirmed by other studies that showed low cerebrospinal fluid NGF levels [18, 22]; other authors observed instead normal NGF values in cerebrospinal fluid and in serum [25].

Statistical analysis of the two groups of Rett patients showed for the first time in humans a link between low NGF levels and the alteration of ventricular repolarization expressed by QTc prolongation, which was established by previous experimental evidence [21, 24] in rats treated with antibodies anti-NGF. QTc interval is considered a predictor of ventricular arrhythmias and other adverse events in various cardiac disease [10]. The alteration of NGF levels, observed in Rett patients, may explain the presence of an altered ventricular repolarization and the consequent higher risk of cardiac arrhythmias.

References

1. Abe T, Morgan DA, Gutterman DD (1997) Protective role of nerve growth factor against postischemic dysfunction of sympathetic coronary innervation. *Circulation 95*:213–220
2. Bracci-Laudiero L, Aloe L, Levi-Montalcini R, et al. (1992) Multiple sclerosis patients express increased levels of β-nerve growth factor in cerebrospinal fluid. *Neurosci Lett 147*:9–12
3. Braddock SR, Braddock BA, Graham JM (1993) Rett syndrome. *Clin Pediatr 32*:613–626
4. Calamandrei G, Aloe L, Hayek J, Zappella M (2001) Developmental profile of serum nerve growth factor levels in Rett complex. *Ann Ist Super Sanita 37*:601–605
5. Cao JM, Chen LS, Ken Knight BH, et al. (2000) Nerve sprouting and sudden cardiac death. *Circ Res 86*:816–821
6. Clegg DO, Large TH, Bodary SC, Reichart LF (1989) Regulation of nerve growth factor mRNA levels in developing rat heart ventricle is not altered by sympathectomy. *Dev Biol 134*:30–37
7. De Bona C, Zappella M, Hayek G, et al. (2000) Preserved speech variant is allelic of classic Rett syndrome. *Eur J Hum Genet 8*:325–330
8. Donovan MJ, Lin MI, Wiegn P, et al. (2000) Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. *Development 127*:4531–4540
9. Driscoll DJ, Edwards WD (1985) Sudden unexpected death in children and adolescents. *J Am Coll Cardiol 5*:118B–121B
10. Elming H, Brendorp B, Kober L, Sahebzadah N, Torp-Petersen C (2002) QTc interval in the assessement of cardiac risk. *Cardiol Electrophysiol Rev 6*:289–294
11. Funck Brentano C, Jailon P (1993) Rate corrected QT interval: techniques and limitations. *Am J Cardiol 72*:17B–23B
12. Garson A (1993) How to measure the QT interval—Wath is normal? *Am J Cardiol 72*:14B–16B
13. Guideri F, Acampa M, Hayek G, Zappella M, Di Perri T (1999) Reduced heart rate variability in patients affected with Rett syndrome. A possible explanation for sudden death. *Neuropediatrics 30*:146–148
14. Hagberg B (1985) Rett's syndrome: prevalence and impact on progressive severe mental retardation in girls. *Acta Paediatr Scand 74*:405–408
15. Hagberg B, Witt-Engerström I (1986) Rett syndrome: a suggested staging system for describing impairment profile with increasing age toward adolescence. *Am J Med Genet 24*:47–59
16. Kaye DM, Vaddadi G, Gruskin SL, Du XJ, Esler MD (2000) Reduced myocardial nerve growth factor expression in human and experimental heart failure. *Circ Res 86*:80–84
17. Kerr AM, Armstrong DD, Prescott RJ, Doyle D, Kearney DL (1997) Rett syndrome: analysis of deaths in the British survey. *Eur Child Adolesc Psychiatr 6*:Suppl 171–74
18. Lappalainen R, Lindholm D, Riikonen R (1996) Low levels of nerve growth factor in cerebrospinal fluid of children with Rett syndrome. *J Child Neurol 11*:296–300
19. Lipani JD, Bhattacharjee MB, Corey DM, Lee DA (2000) Reduced nerve growth factor in Rett syndrome postmortem brain tissue. *J Neuropathol Exp Neurol 59*:889–895
20. Lockhart ST, Turrigiano GG, Birren SJ (1997) Nerve growth factor modulates synaptic transmission between sympathetic neurons and cardiac myocytes. *J Neurosci 17*:9573–9582
21. Malfatto G, Rosen TS, Steinberg SF, et al. (1990) Sympathetic neural modulation of cardiac impulse initiation and repolarization in the newborn rat. *Circ Res 66*:427–437
22. Riikonen R, Vanhala R (1999) Levels of cerebrospinal fluid nerve growth factor differ in infantile autism and Rett syndrome. *Dev Med Child Neurol 41*:148–152
23. Sekul EA, Moak JP, Schultz RJ, et al. (1994) Electrocardiographic findings in Rett syndrome: an explanation for sudden death? *J Pediatr 125*:80–82
24. Sun LS, Legato MJ, Rosen TS, Steinberg SF, Rosen MR (1993) Sympathetic innervation modulates ventricular impulse propagation and repolarization in the immature rat heart. *Cardiovasc Res 27*:459–463
25. Vanhala R, Korhonen L, Mikelsaar M, Lindholm D, Riikonen R (1998) Neurotrophic factors in cerebrospinal fluid and serum of patients with Rett syndrome. *J Child Neurol 13*:429–433
26. Weskamp G, Otten U (1987) An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and peripheral tissues. *J Neurochem 48*:1779–1786