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Central and systemic C-type Natriuretic Peptide are both reduced in Parkinson's Disease

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ABSTRACT

Introduction: C-type Natriuretic Peptide is a neuropeptide widely expressed in the central nervous system including dopaminergic neurons projecting to basal ganglia. Previous work shows that concentrations of the peptide in cerebrospinal fluid are depressed in drug naïve PD subjects, decline over time and can be restored by doses of monoamine oxidase inhibitors that delay the need for levodopa. Whether plasma levels are similarly depressed in drug naïve subjects, or affected by dopaminergic drugs, is unknown. Our objectives were to determine whether (i) peptide products in plasma differ from normal in PD, and (ii) levels are affected by dopaminergic treatment.

Methods: Plasma C-type Natriuretic Peptide and amino-terminal proCNP were measured in two groups – 27 drug naïve subjects with PD, and 30 subjects stabilized on dopaminergic drugs for at least 3 years. Values were compared with standard deviation scores from a population reference group without neurological disorder. Independent associations with predetermined variables known to affect plasma concentrations were assessed by multivariate analysis.

Results: In both PD groups, plasma amino-terminal proCNP was significantly depressed compared to the reference range. Concentrations did not differ between the two groups. No correlation with disease duration or phenotype was found. Across all subjects, in a model initially comprising 7 factors, serum creatinine, PD and age were independent significant associations with amino-terminal proCNP.

Conclusions: Plasma concentrations of amino-terminal proCNP are depressed in PD, are likely to result from diminished reabsorption from central sources, and may be useful in monitoring onset and effects of therapeutic interventions in PD.

1. Introduction

Extensive studies seeking biological markers of the onset and disease progression in Parkinson Disease (PD) reveal multiple biochemical changes in a variety of tissue fluids [1,2] but none has yet proved useful in the clinic when measured in blood. Recently we showed that a product of the C-type Natriuretic Peptide (CNP) gene expression, amino-terminal proCNP (NTproCNP), is reduced in cerebrospinal fluid (CSF) drawn from untreated PD subjects, declines over time and shows concordant increase with clinical responses to treatment with mono amine oxidase inhibitors [3]. CNP belongs to a family of closely related peptides best known for actions within the cardiovascular system. However CNP is distinct in having a unique receptor, in lacking natriuretic activity and (in contrast to other family members) in having largely paracrine actions which regulate cell proliferation and differentiation in a variety of tissues [4]. Within the central nervous system (CNS), CNP and its specific receptor are widely expressed throughout the brain and spinal cord [5–7] in mammals including primates [8]. Although actions within the intact nervous system remain to be clarified, numerous studies support CNP's role in neural growth and connectivity [9] within brain and axonal entry zones of cranial sensory and dorsal root ganglial neurons [10]. Further, CNP pathway activity co-locates with dopaminergic neurons in sub thalamic nuclei and basal ganglia [11] including neurons expressing tyrosine hydroxylase [12]. In this context, the finding that NTproCNP in PD is reduced in CSF, declines with time and can be restored by monoamine oxidase inhibitors [3] supports the view that the peptide is modulated within the CNS in PD and may confer neuroprotection.

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Whether concentrations of NTproCNP in the systemic circulation in untreated PD are abnormal is unknown but important to address in the light of CNP's possible roles in causation and/or signalling disease progression. Of note, in a small study of 11 male subjects with early onset PD – all receiving dopaminergic drugs – we observed that the concentrations of NTproCNP in plasma were unexpectedly low when compared to a reference range established in well adult volunteers recruited from the electoral roll. In contrast, others report that plasma NTproCNP is raised in subjects with long established PD receiving dopaminergic therapy [13] – findings that were attributed to potential pro-inflammatory actions of these drugs. To clarify the status of CNP products in the systemic circulation in PD we have now examined phenotype, plasma NTproCNP and the bioactive form proCNP82-103 (CNP) in (i) drug naïve subjects (group N) and (ii) a similar group stabilized on antiparkinsonian therapy for at least 3 years (group D). On the basis of our previous findings [3], we hypothesised that plasma NTproCNP would not differ between the 2 groups but would be reduced in both groups when compared to values determined in an age and gender matched group of well subjects without neurological disorder. We further postulated that associations of peptides with phenotype in PD subjects would not be significant.

2. Methods

Approval was granted by the Ethics Committee of New Zealand (14/CEN/169/AM01), the Canterbury District Health Board Local Authority and Te Komiti Whakarite. All subjects gave informed written consent for a separate clinical assessment and blood test.

Subjects were recruited from Neurology outpatient clinics in the Canterbury region, New Zealand. All subjects had a clinical diagnosis of Idiopathic Parkinson Disease fulfilling UK Brain Bank Criteria [14] and were between the ages of 18–80 years. The drug naive group (Group N) had no history of exposure to dopaminergic therapy or monoamine oxidase inhibitors. Those on dopaminergic therapy (Group D) had been exposed for at least 3 years. Mean levodopa equivalent daily dose for each subject was determined as per Tomlinson et al. [15]. Exclusion criteria were significant cardiovascular, renal or other CNS disorder, insulin dependent diabetes, creatinine exceeding 150 μmol/L or the inability to give consent. Assessments were performed in most cases by the Principal Investigator (a senior Neurology trainee), and in two cases by Research Assistants with experience in PD research. Disease duration was established on history with corroboration where available from clinical records. In order to measure disease phenotype and severity, a formulaic clinical history and examination was conducted, comprising UPDRS parts II and III, Non-Motor Symptoms Assessment Scale for Parkinson Disease (NMSQ), Montreal Cognitive Assessment (MoCA) and Modified Hoehn and Yahr (H&Y) staging. History relating to the frequency (1 = rare; 2 = less than one episode per week; 3 = greater than one per week but less than 7; 4 = daily episodes) of episodic (symptomatic) orthostatic hypotension (OH) and severity (1 = mild "light headed" requiring no action; 2 = moderate, symptoms requiring change in posture; 3 = severe, actual faint or requiring supine posture) was sought and scored separately in each subject. Blood pressure was measured manually in the sitting position.

Blood samples were drawn into Heparin and EDTA tubes on wet ice, then rapidly transported to the Christchurch Heart Institute Laboratory for processing. After centrifugation, samples for CNP products were stored at minus 80 C prior to assay.

Participants for the reference range were well adults recruited from the electoral roll of Canterbury, New Zealand. After a brief examination, subjects with history or any sign of heart disease, renal impairment or neurological disorder were excluded [16]. Because plasma concentrations of both CNP products are affected by age and gender, individual values in all subjects in the current study were transformed to standard deviation scores (SDS) by the LMS procedure [17].

2.1. Assays

The CNP gene (*NPPC*) codes for a propeptide (proCNP) of 103 amino acids which is then processed intracellularly to the bioactive form (CNP 53, or 51–103 proCNP) and an inactive aminoterminal fragment (NTproCNP, or 1–50 proCNP). Both peptides are presumed to be co-secreted in equimolar amounts but CNP 53 is further cleaved to CNP 22 (proCNP 82–103) at unknown sites and in health only trivial amounts enter the circulation. Because CNP 53 and CNP22, unlike NTproCNP, are rapidly degraded at source, the concentration of NTproCNP is more stable, is easily measured in extracellular fluids and therefore considered to reflect levels of *NPPC* expression more reliably than does bioactive CNP [18] provided renal function is normal. Plasma CNP and NTproCNP were measured by radioimmunoassay as previously described [19], after extraction over SepPac cartridges (Waters Corp., Milford, MA). Intra- and inter assay coefficients of variations were as follows: CNP (5.0% and 8.5% at 8.1 pmol/L) and NTproCNP (6.6% and 8.1% at 45 pmol/L). Serum creatinine was determined by the Architect c8000 analyser (Abbott Laboratories, Abbott Park IL USA).

2.2. Statistical analyses

All values are presented as mean ± SEM. Data was log transformed where appropriate to satisfy parametric assumptions. Student t-tests were used to compare group N and D indices. Spearman rank coefficient was used to determine correlations between variables, presented as r values. Multiple linear regression analysis was performed using a backward stepwise approach considering factors that were significant at $r > 0.2$ level by univariate analysis. All analyses were performed using SPSS version 22 (SPSS Inc. Chicago, IL). Statistical significance was assumed when $p < 0.05$.

3. Results

Twenty seven subjects in group N, and 30 in group D were recruited and completed the study. One group D subject with an elevated serum creatinine (176 μmol/L) was excluded from further analysis. Data collection was complete except the MoCA score in one subject in group N was unavailable and a single entry (MoCA, and UPD II and III) affecting 3 separate subjects was not available for phenotyping in Group D. Mean duration of disease (from onset of symptoms) was 13.1 years and 3.1 years in groups D and N respectively. Details of drugs in those taking dopaminergic therapy (group D) are shown in the Table (**Supplementary material**). All were receiving levodopa at the time of sampling. Five of these received only levodopa; 17 received levodopa plus a dopamine agonist; 1 received levodopa plus a MAO inhibitor; 6 received levodopa plus both a dopamine agonist and MAO inhibitor. Among all PD subjects, orthostatic hypotension (OH) was scored as moderate or severe in 11, and in 8 subjects its frequency was at least once weekly.

3.1. Differences between groups

Mean age did not differ significantly between the two groups. While more males were recruited, the gender distribution was similar in groups N and D. As shown in Table 1, mean H&Y score was higher and the MoCA lower ($p > 0.001$) in group D, consistent with the longer duration of disease but UPDRS scores did not differ. In

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