Diurnal Plasma Concentrations of Natriuretic Propeptides in Healthy Young Males

To the Editor:

Natriuretic peptides are a family of structurally related hormones encoded by genes for atrial natriuretic peptide (ANP),\(^1\) B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). Although the bioactive peptides are related, their tissue and receptor specificities differ. Natriuretic peptide production can also be assessed by measuring the N-terminal fragments from their prohormones in plasma, because these fragments constitute stable markers in vitro and in vivo. The molecular heterogeneity of the propeptides has proved to be complex, and cardiac biosynthesis involves both endoproteolytic cleavage and posttranslational modification \((1)\). The BNP precursor, for instance, is a glycosylated polypeptide and variably matures to the C-terminal, bioactive BNP hormone. The CNP products encoded by the NPPC\(^2\) (natriuretic peptide C) gene have been difficult to measure accurately because their plasma concentrations are considerably lower than for the ANP and BNP peptides.

Diurnal hormone production is a hallmark of many endocrine systems. In mouse models, expression of the genes encoding ANP and BNP has been shown to be regulated in a circadian manner by clock genes. We recently reported that BNP mRNA contents, but not ANP mRNA contents, are produced in a circadian manner in the ventricles of wild-type mice and that the production pattern reflects the local expression of the central clock genes Per1 [period homolog 1 \((Drosophila)\)] and Bmal1 [officially known as Arntl (aryl hydrocarbon receptor nuclear translocator-like)] \((2)\). In the present study, we included 24 healthy Caucasian male volunteers (mean age, 26 years). The study was approved by the local ethics committee, and the demographic data of the volunteers and the study setup have previously been reported \((3)\). For proANP measurement, we used 2 immunoassays. The first assay measures midregional proANP (MR-proANP) on the Kryptor platform \((BRAHMS)\). The assay sensitivity is 6 pmol/L with an inter assay CV of 20% at 18 pmol/L and 10% at 65 pmol/L \((4)\). In addition, we used a newly developed assay for quantifying “total” proANP in plasma. In brief, this assay uses a preanalytical step with proteolytic plasma cleavage, which releases an N-terminal 1–16 fragment from proANP. The 1–16 fragment is then quantified with an RIA directed against the C terminus \((5)\). The inter assay CV is 11.0% at 240 pmol/L and 6.0% at 2468 pmol/L; the limit of detection is 34 pmol/L. N-terminal proBNP (NT-proBNP) was analyzed on a Modular E system \((Roche Diagnostics)\); the level of detection is 0.6 pmol/L with an inter assay CV of 6.0% at 10.2 pmol/L and 5.0% at 45 pmol/L. The functional sensitivity was 4.9 pmol/L with a CV of 20%. Finally, total proCNP was measured with an in-house assay. The analytical sensitivity was 3.2 pmol/L, and the inter assay CV was 12.9% at 8 pmol/L and 6.1% at 40 pmol/L. The time-related data for all volunteers were analyzed for the presence of diurnal changes with the methods for cosinor rhythmometry for groups, as described previously \((3)\). Results are presented as the mean \((SD)\) after normal distribution of the data was verified with the D’Agostino–Pearson omnibus and Shapiro–Wilk normality tests.

The variation in the plasma melatonin concentration validated the regular routine of diurnal activity and nocturnal sleep, with concentrations showing the expected 24-h pattern for the group. Concentrations were lowest in the afternoon and increased gradually to peak concentrations at 0334 h \((P < 0.0001)\). There was a distinct diurnal pattern for MR-proANP and “total” proANP plasma concentrations \((Fig. 1)\), with the lowest concentrations occurring during late afternoon \([mean \text{ MR-proANP at 1800 h, 30.4 (2.3) pmol/L}]\) and the highest concentrations occurring at night \([mean \text{ MR-proANP at 0300 h, 43.5 (2.9) pmol/L}]\). The proANP plasma profile thus mimicked the melatonin profile. In contrast, no significant change over a 24-h period could be established for plasma NT-proBNP and total proCNP concentrations \([mean \text{ NT-proBNP, 1.8 (0.1) pmol/L; mean total proCNP, 46.2 (0.3) pmol/L; } n = 216 \text{ for both} \). Our results of diurnally varying proANP plasma concentrations but nondiurnal NT-proBNP plasma concentrations in healthy individuals seem to conflict with previous findings regarding the production of mRNA in mouse hearts \((2)\). That study noted a circadian production of BNP mRNA in the cardiac ventricles but little response for the ANP mRNA content in the atria. This discrepancy may partially be explained by differences between mice and humans in gene expression, given that normal ventricular expression of the gene encoding BNP is mostly a rodent phenomenon. Moreover, the differences suggest that mRNA data and plasma concentrations may not always be correlated and that the cellular pattern of secre-
Fig. 1. Plasma profiles for MR-proANP and processing-independent (PIA) (or “total”) proANP (A), NT-proBNP (B), and total proCNP (C) over a 24-h period in 24 healthy young males. Shaded area represents the nighttime/early-morning period.
tion from cardiomyocytes should also be considered. Finally, rodents are not subjected to hemodynamic changes related to sleeping, whereas humans go from an upright to a horizontal position, thereby increasing venous return.

We studied the diurnal plasma profile of the entire natriuretic propeptide family. By measuring unique epitopes in the precursor structures, we show that proANP, but not NT-proBNP and total proCNP, circulates in a diurnal manner, which suggests that the circadian sodium homeostasis reported in healthy individuals may partly be regulated by cardiac ANP production (6). A diurnal variation could have implications with respect to the clinical use of MR-proANP measurement in population studies and/or for diagnosis/risk assessment in healthy individuals. Our findings also suggest that the biological variation for proANP may relate to diurnal production to some extent, whereas the biological variation in NT-proBNP plasma concentration seems caused by other stimuli.

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