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Dopamine concentration in blood platelets is elevated in patients with head and neck paragangliomas

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Abstract

Background: Plasma 3-methoxytyramine (3-MT), a metabolite of dopamine, is elevated in up to 28% of patients with head and neck paragangliomas (HNPGLs). As free dopamine is incorporated in circulating platelets, we determined dopamine concentration in platelets in patients with a HNPGL.

Methods: A single center cohort study was performed between 2012 and 2014. Thirty-six patients with a HNPGL were compared to healthy controls (68 for dopamine in platelets and 120 for plasma 3-MT).

Results: Dopamine concentration in platelets was elevated in HNPGL patients compared to healthy controls (median [interquartile ranges] 0.48 [0.32–0.82] pmol/10^9 platelets vs. 0.31 [0.24–0.47] pmol/10^9 platelets; $p<0.05$), whereas plasma 3-MT concentration did not differ between both groups (0.06 [0.06–0.08] nmol/L vs. 0.06 [0.06–0.06] nmol/L; $p=0.119$). Based on 68 healthy controls, the reference interval for dopamine concentration in platelets was 0.12–0.97 pmol/10^9 platelets. Six (16.7%) patients with a HNPGL demonstrated an increased dopamine concentration in platelets compared to three (8.3%) patients with an increased plasma 3-MT level ($p=0.053$). The sensitivity and specificity were 16.7% and 98.5% for platelet dopamine and 8.3% and 97.5% for plasma 3-MT concentration ($p=0.37$).

Conclusions: Dopamine concentration in platelets is elevated in patients with a HNPGL compared to healthy subjects, and may be a novel biomarker for dopamine producing paraganglioma.

Keywords: 3-methoxytyramine; dopamine; paraganglioma; platelets.

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Introduction

Paraganglioma of the head and neck (HNPGL) are rare neuroendocrine tumors that arise from the parasympathetic nerve system [1]. HNPGLs may occur sporadically but are also associated with germline mutations mainly in genes encoding succinate dehydrogenase (SDH) subunit B and D (*SDHB*, *SDHD*) [2, 3]. HNPGLs are most often benign tumors with a slow growth rate of about 1–2 mm/year [4, 5].

The biochemical diagnosis of sympathetic paraganglioma and pheochromocytoma is based on the demonstration of elevated metanephrines in plasma and/or urine [6]. In contrast, elevated metanephrines can be detected only in a minority of patients with a HNPGL [7]. Therefore, there is a need for novel biochemical tests that would be useful for diagnosing HNPGLs, and for the follow-up of these patients. Ideally, such a test would also facilitate early detection of a HNPGL, especially in *SDHx* mutation carriers. Previous studies have demonstrated that HNPGLs are able to synthesize dopamine. Excess

dopamine secretion, measured as elevated dopamine and/or its 3-O-methylated metabolite 3-methoxytyramine (3-MT) in urine and/or plasma, was reported to be present in 19%–28% of patients with a HNPGL [7–9].

Nearly all circulating dopamine is stored in platelets, whereas reportedly only 1% circulates free in plasma [10]. Free dopamine is rapidly incorporated by circulating platelets through the dopamine transporter (DAT) [11]. As the lifespan of platelets is 8–10 days, the platelet dopamine concentration is a reflection of the dopamine secretion in the past 10 days [11, 12]. This offers the theoretical advantage that intermittent secretion of dopamine by HNPGLs might be better detected by measurement of its concentration in platelets.

For this report we tested the extent to which measurement of dopamine concentration in platelets represents a marker of dopamine overproduction. To this end we compared dopamine concentration in platelets with the dopamine metabolite 3-MT in plasma in patients with a HNPGL.

Patients and methods

Study population

For this single center study, we included consecutive patients older than 18 years of age diagnosed with a HNPGL, who visited the outpatient clinic of the Department of Endocrinology and Metabolic diseases or the Department of Ear Nose and Throat at the University Medical Center Groningen between February 2012 and February 2014. We excluded patients using selective serotonin reuptake inhibitors (SSRIs) or tricyclic antidepressants (TCAs), because these medications might decrease the uptake of dopamine in platelets [13, 14]. In addition, dopamine can be depleted from platelets by blocking the dopamine uptake through the DAT by use of methylphenidate or illicit drugs such as amphetamine and cocaine [15, 16]. We therefore excluded patients reporting the use of these substances. We also excluded patients with concurrent sympathetic paraganglioma or pheochromocytoma.

The presence of a HNPLG was based on a combination of anatomical imaging CT and/or MRI and/or functional imaging including ^{111}In-octreotide scintigraphy (octreoscan), ^{123}I-metaiodobenzylguanidine (MIBG scintigraphy) and/or 6-[^{18}F]-fluoro-L-3,4-dihydroxyphenylalanine (DOPA) positron emission tomography (PET) (^{18}F-DOPA PET) [17].

Patients were seen by a (research) physician (T.E.O., A.N.A.v.H.S., M.N.K., R.P.F.D., T.P.L.), with specific attention to symptoms, medication use and family history. Blood pressure and pulse rate were manually measured while sitting. Hypertension was defined as a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg or the use of anti-hypertensive medication. The medical history and results of germline mutation analyses were retrieved from patient's medical files. Blood samples for dopamine in platelets and plasma free metanephrine (MN), normetanephrine (NMN) and 3-MT were collected simultaneously from patients in sitting position without prior dietary restrictions [18].

The research was conducted in accordance with the Declaration of Helsinki Principles and the study protocol was approved by the Medical Ethics Committee of the University Medical Center of Groningen. Both patients and healthy controls gave written informed consent.

Reference population

Reference values for plasma free 3-MT concentrations were determined in 120 healthy controls (63 men and 57 women, 36–81 years of age) participating in the Prevention of Renal and Vascular End Stage Disease (PREVEND) study in sitting position without prior dietary restrictions [19].

Reference values for dopamine in blood platelets were established in a group of 68 healthy controls (35 men and 33 women, 35–56 years of age). These healthy volunteers were recruited via the Department of Medical Oncology at the University Medical Center of Groningen.

Analytical methods

Blood samples were collected by venipuncture in two 10 mL Vacutainer Tubes (Becton Dickinson) containing K_2EDTA solution as anticoagulant. To determine the dopamine concentration in blood platelets, plasma blood samples were centrifuged at 120 g for 30 min at an ambient temperature to get platelet rich plasma (PRP) within 1 h after sample collection. In addition, a platelet count of PRP was determined before storing the samples at -80 °C until processing at the department of laboratory medicine. Glutathion was added as an antioxidant. PRP was transferred to storage tubes using plastic pipettes.

After thawing, deuterium labeled dopamine was added as internal standard to PRP. Samples were subsequently derivatized, essentially as described by van de Merbel et al. [20]. After derivatization, samples were extracted and analyzed using solid phase extraction in combination with isotope dilution tandem mass spectrometry, essentially as described by van de Merbel et al. [20]. Plasma free serotonin was measured as an internal control for the integrity of the platelets. Results of platelet dopamine were obtained by dividing PRP dopamine concentration by the previously obtained platelet count. The intra-assay and inter-assay analytical variation coefficients were <5% and 12%, respectively. The lower limit of quantification was 15 pmol/L.

Plasma free MN, NMN and 3-MT assays were performed with a High-Performance Liquid Chromatography tandem mass spectrometric technique (LC-MS/MS) with automated solid phase extraction sample preparation, as previously described by de Jong et al. [19]; the only modification was that the chromatography was optimized, so 3-MT was chromatographically separated from MN to prevent ionic cross talk as described by Twentyman et al. [21, 22]. Established reference intervals for plasma free metanephrines were: MN 0.07–0.33 nmol/L, NMN 0.23–1.07 nmol/L, 3-MT <0.17 nmol/L [19]. The lower limit of quantification of plasma 3-MT was 0.06 nmol/L. The intra-assay and inter-assay analytical variation coefficients were 2.5%–4.8% and 3.4%–5.6% for free plasma MN, 5.1%–6.2% and 4.2%–7.1% for free plasma NMN, and 4.4%–8.0% and 4.5%–11.1% for free plasma 3-MT, respectively.

Sample size calculation

Urinary or plasma 3-MT is increased in 19%–28% of patients with a HNPGL [7–9]. Assuming that an additional 16.6% of HNPGL patients would have an abnormal test result when measuring dopamine concentration in blood platelets instead of plasma 3-MT (i.e. one in every 6 patients, which was deemed clinically relevant), the sample size that would be required to provide more than 80% power with a two-sided alpha-level of 0.05 was calculated to be 30 HNPGL patients (McNemar test).

Statistical analysis

Data are presented as mean±standard deviation (SD) or as median with interquartile ranges [IQR] where appropriate. Relationships between dopamine in platelet concentration and plasma free 3-MT were evaluated by Spearman's rank correlation analysis (Spearmans ρ). Reference intervals for platelet dopamine concentrations were calculated using EP Evaluator™ software. Differences between HNPGL patients and healthy controls for dopamine concentration in platelets and plasma free 3-MT were evaluated using the Mann-Whitney U-test. Based on the calculated reference values we calculated the sensitivity and specificity of both markers. With these reference values we also calculated the number of patients who had an elevated dopamine concentration in platelets and plasma free 3-MT and used a χ^2 test for comparison. To test for a difference in sensitivity and specificity between dopamine concentration in platelets and plasma 3-MT, we used a McNemar test. Statistical analyses were performed with PASW statistics (version 22; IBM/SPSS, Armonk, New York, USA). A two sided p-value <0.05 was considered statistically significant.

Table 1: Patient characteristics.

	HNPGL (n=36)
Sex (male/female)	13/23
Age, years (mean±SD)	56±18
Tumor size [cm³±IQR]	2.1 [0.20–21]
Tumor location	
Carotid body PGL	23
Jugulotympanic PGL	17
Vagal PGL	1
Multifocal PGL	4
Germline mutations, n (%)	
None (sporadic)	14 (39)
Unknown	5 (14)
Familiar Syndrome	17 (47)
VHL	1 (3)
SDHA	1 (3)
SDHB	10 (28)
SDHD	4 (11)
SDHAF2	1 (3)
Complaints, n (%)	
Headache	12 (33)
Palpitations	12 (33)
Perspiration	10 (28)
Pallor	3 (8)
Nausea	8 (22)
Flushes	8 (22)
Tiredness	14 (39)
Anxiety attacks	3 (8)
Tinnitus	18 (49)
Impaired hearing	19 (52)
Vertigo	12 (33)
Dysphagia	8 (22)
Hoarseness	7 (19)
Hemodynamic control	
Mean BP (±SD, mm Hg)	142 (±22)/83 (±8)
Mean pulse (±SD, beats/min)	71 (±12)
Hypertension, n (%)	24 (58)

HNPGL, Head and neck paraganglioma; y, year; SD, standard deviation; IQR, interquartile range; VHL, von Hippel Lindau; SDHB, succinate dehydrogenase subunit B; SDHD, succinate dehydrogenase subunit D; SDHAF2, succinate dehydrogenase assembly factor 2; BP, blood pressure.

Results

Patient characteristics

Between February 2012 and February 2014, 36 HNPGL patients were included. Table 1 shows the characteristics of the participants included in the study. Free plasma MN and NMN concentrations were in the normal range for both patients with a HNPGL and healthy controls. Plasma free NMN concentrations were significantly higher in patients with a HNPGL, 0.77 [0.59–1.02] nmol/L, compared with healthy controls 0.53 [0.41–0.70] nmol/L (p<0.001) (Table 2).

Dopamine in platelets and plasma free 3-MT in HNPGL patients

The median value of dopamine in blood platelets was significantly higher in patients with a HNPGL, 0.48 [0.32–0.82] pmol/10^9 platelets, compared with healthy controls 0.31 [0.24–0.47] pmol/10^9 platelets (p<0.05) (Table 2 and Figure 1). Plasma free 3-MT concentrations in patients with a HNPGL, 0.06 [0.06–0.08] nmol/L, were not significantly different from concentrations in healthy controls 0.06 [0.06–0.06] nmol/L (p=0.12) (Table 2 and Figure 1). The median free plasma dopamine concentrations were not significantly different between patients with a HNPGL and healthy controls (p=0.09) (Table 2 and Figure 1).

Based on 68 healthy controls, the calculated reference interval for dopamine concentration in platelets was 0.12–0.97 pmol/10^9 platelets. When using this reference

Table 2: Dopamine levels in platelets compared with plasma free metanephrine, normetanephrine and 3-methoxytyramine (3-MT).

	HNPGL (n=36)	Controls
Plasma free 3-MT, nmol/L	0.06 [0.06–0.08]	0.06 [0.06–0.06][a]
Plasma free NMN, nmol/L	0.77 [0.59–1.02][c]	0.53 [0.41–0.70][a]
Plasma free MN, nmol/L	0.21 [0.12–0.27]	0.18 [0.13–0.23][a]
Dopamine in platelets, pmol/10^9 platelets	0.48 [0.32–0.82][d]	0.31 [0.24–0.47][b]
Plasma free dopamine, nmol/L	55.6 [44.9–68.1]	51.1 [39.0–59.4][b]

[a]Plasma free metanephrine, normetanephrine and 3-methoxytyramine levels of the reference population (n=120). [b]Dopamine concentrations in platelets and plasma free dopamine concentrations determined in healthy controls (n=68). Values are reported as median [interquartile range] or mean (±SD). [c]p<0.001 and [d]p<0.05 compared with patients with a HNPGL. NMN, Normetanephrine; MN, metanephrine; 3-MT, 3-methoxytyramine.

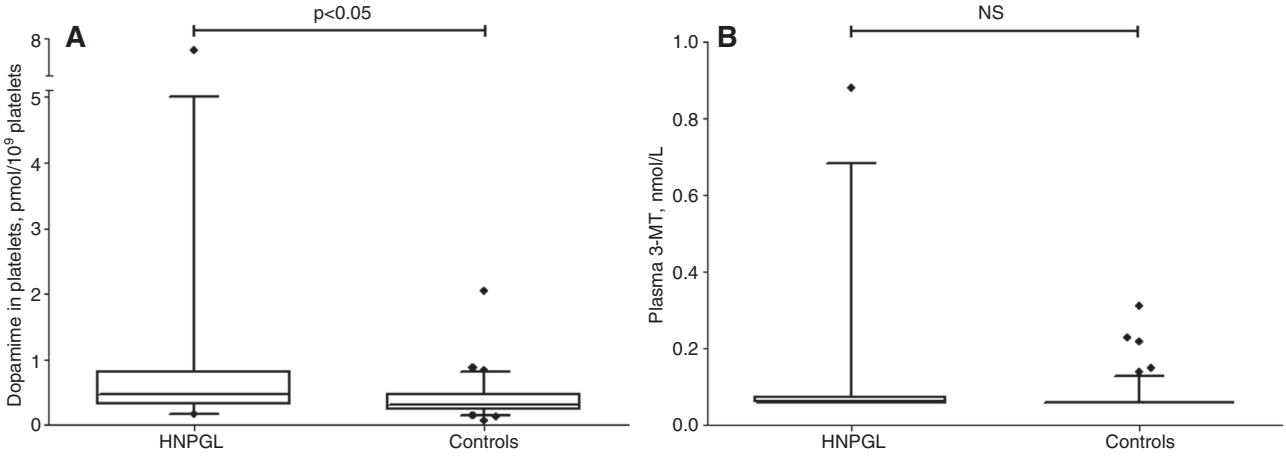

Figure 1: Box plots whiskers representing 5 and 95 percentiles and outliers.
Concentrations of platelet dopamine (A) and plasma free 3-methoxytyramine (3-MT) (B) in patients with head and neck paraganglioma with a HNPGL and healthy controls.

interval, 6 (16.7%) patients with a HNPGL had an increased dopamine concentration in platelets. In contrast, only three (8.3%) HNPGL patients showed an elevated plasma free 3-MT level (i.e. ≧0.17 nmol/L) (p=0.053). The calculated sensitivity and specificity were 16.7% and 98.5% for platelet dopamine concentration and 8.3% and 97.5% for plasma 3-MT concentration (p=0.37). For patients with HNPGLs, the Spearman's correlation coefficient between dopamine levels in platelets and plasma free 3-MT was 0.19 (p=0.29). Moreover, there was no relation between tumor size and dopamine concentration in platelets (ρ_s=0.05, p=0.79).

Discussion

In the present study, we show for the first time that dopamine concentration in blood platelets is significantly higher in patients with a HNPGL than in healthy controls.

Dopamine concentration in platelets has been measured previously only in patients with schizophrenia and in patients with migraine. Dopamine concentration in platelets is higher in patients with migraine compared with healthy controls [12, 23–25]. The platelet dopamine concentration decreases during a migraine attack, which is accompanied by an increase of the plasma dopamine concentration [25, 26]. In patients with schizophrenia, dopamine uptake in platelets did not differ from healthy controls [23].

Theoretically, measurement of platelet dopamine may represent a more sensitive method for detection of dopamine overproduction, because it is presumed to reflect the average exposure to plasma free dopamine during the preceding 8–10 days [27]. The transport of dopamine into the platelet is facilitated by the DAT [11]. Depending on the orientation of DAT (inward- or outward-facing), dopamine can be transported either into or out of the platelet. After transportation into the cytoplasm, dopamine is taken up into dense granules by the vesicular monoamine

transporter (VMAT) 2 until it is released by exocytosis (Figure 2) [28–30]. In this way platelets can take up circulating dopamine and serotonin released from the autonomic nerve endings and from dopamine secreting organs [11].

We demonstrate for the first time in patients with HNPGLs that dopamine in human blood platelets can be measured with sufficient accuracy, as the applied measurement has sufficient analytical sensitivity and specificity to reproducibly measure the analyte both in healthy controls and patients. Our report shows an elevated dopamine concentration in platelets from a relevant proportion of HNPGL patients and should be regarded as a proof of concept study. Increased plasma free 3-MT concentrations were already demonstrated in previous studies. In our population, the dopamine concentration in platelets was more often increased, compared with the plasma free 3-MT concentration, 16.7% vs. 8.3%, respectively. This seems at variance with the study by van Duinen et al. [9], who found an increase of plasma free 3-MT in 28% of their patients with a HNPGL. This difference is probably explained by a difference in study population, as the study by van Duinen et al. [9] consisted predominantly of *SDHx* associated mutation carriers (95 out of 124; 77%), while in our study only 16 out of 36 had a *SDHx* associated mutation (44%). Dopamine production has been shown to be particularly prevalent among *SDHB* and *SDHD* mutation carriers, compared with *SDHx* negative patients [31]. This could be the result of an indirect stimulatory effect of *SDHx* mutations on the tyrosine hydroxylase enzyme activity, which is the rate-limiting enzyme in the dopamine synthesis pathway [32, 33].

Although dopamine concentrations in platelets were significantly higher in HNPGL patients than in healthy controls, their diagnostic sensitivity was not significantly different from plasma free 3-MT. This could also be the consequence of our power analysis, as the percentage of patients with an increased plasma free 3-MT concentration was found to be lower than we expected. It should, therefore, be emphasized that further studies are needed to evaluate whether the combined measurement of dopamine in platelets and free 3-MT in plasma, both with high diagnostic specificity but with apparently low sensitivity, would improve biochemical characterization of patients with a suspected HNPGL. Specifically, the usefulness of measurement of dopamine in platelets in the (post-treatment) follow-up of HNPGL patients, as well as in the characterization of *SDHx* mutation carriers, must still be

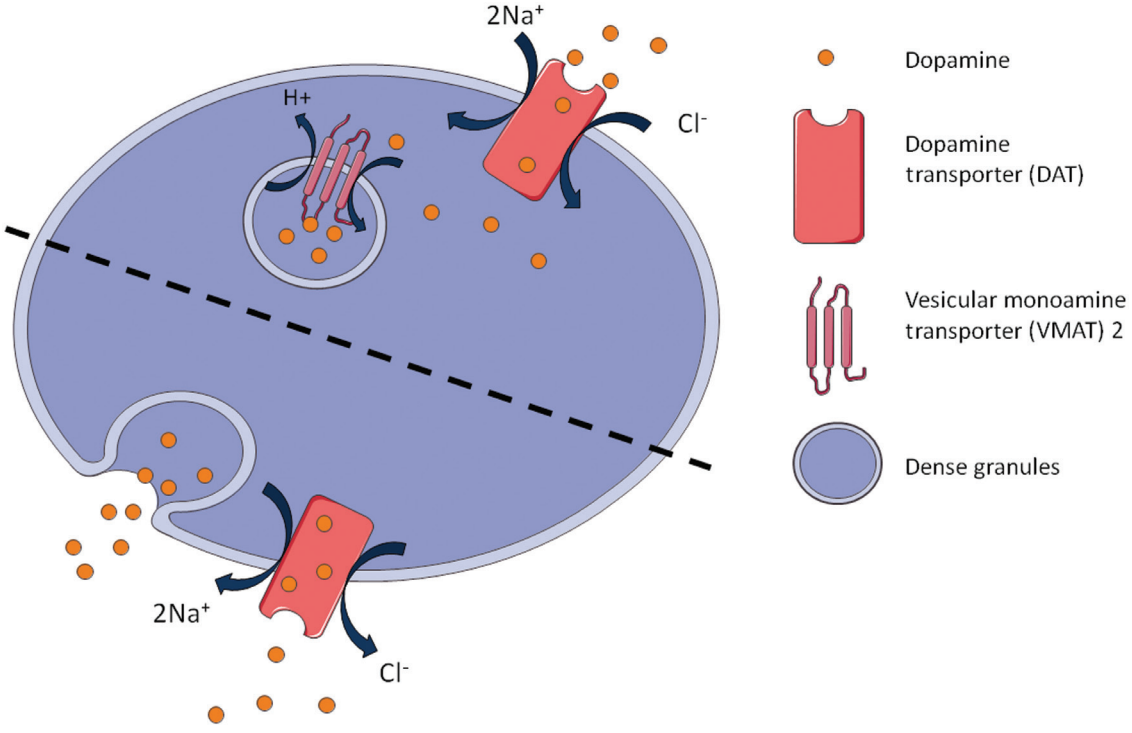

Figure 2: Uptake and exocytosis of dopamine in platelets.
The transport of dopamine into the platelet is facilitated by the dopamine transporter (DAT). After transportation into the cytoplasm, dopamine is taken up by dense granules through the vesicular monoamine transporter (VMAT) 2 until it is released by exocytosis. (Image constructed using Servier Medical Art)

established. Moreover, dopamine in platelets might also be used as a tumor marker for postsurgical follow-up in patients with a HNPGL or pheochromocytoma.

In addition, we did not find any differences in free plasma dopamine levels between patients and healthy controls. In our study we noticed a higher percentage of plasma free dopamine (31±21%) compared with the previously described 1% by in the study by Da Prada and Picotti [10]. The difference in distribution of dopamine between platelets and plasma between the two studies could be explained by the use of different analytical methods, as we used mass spectrometric detection whereas Da Prada used a radioenzymatic assay. The suitability of our pre-analytical procedure has previously been established for serotonin [34].

In conclusion, platelet dopamine concentrations are increased in patients with a HNPGL compared with healthy controls. Further studies are warranted in order to determine the diagnostic value of the measurement of dopamine concentration in platelets in patients with HNPGL, pheochromocytoma, sympathetic PGL and in *SDHx* mutation carriers.

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