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^{18}F -FDOPA PET/CT Uptake Parameters Correlate with Catecholamine Secretion in Human Pheochromocytomas

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Keywords

Catecholamine · Chromaffin cells · Endocrine tumor · Exocytosis · Pheochromocytoma · ^{18}F -FDOPA PET/CT

Abstract

Background: ^{18}F -FDOPA positron emission tomography/computed tomography (PET/CT) is a sensitive nuclear imaging technology for the diagnosis of pheochromocytomas (PHEO). However, its utility in determining predictive factors for the secretion of catecholamines remains poorly studied.

Methods: Thirty-nine histologically confirmed PHEO were included in this retrospective single-center study. Patients underwent ^{18}F -FDOPA PET/CT before surgery, with an evaluation of several uptake parameters (standardized uptake values [SUV_{max} and SUV_{mean}] and the metabolic burden [MB] calculated as follows: $\text{MB} = \text{SUV}_{\text{mean}} \times \text{tumor volume}$) and measurement of plasma and/or urinary metanephrine (MN), normetanephrine (NM), and chromogranin A. Thirty-five patients were screened for germline mutations in the *RET*, *SDHx*, and *VHL* genes. Once resected, primary cultures of

5 PHEO were used for real-time measurement of catecholamine release by carbon fiber amperometry. **Results:** The MB of the PHEO positively correlated with 24-h urinary excretion of NM ($r = 0.64, p < 0.0001$), MN ($r = 0.49, p = 0.002$), combined MN and NM ($r = 0.75, p < 0.0001$), and eventually plasma free levels of NM ($r = 0.55, p = 0.006$). In the mutated patients (3 *SDHD*, 2 *SDHB*, 3 *NF1*, 1 *VHL*, and 3 *RET*), a similar correlation was observed between MB and 24-h urinary combined MN and NM ($r = 0.86, p = 0.0012$). For the first time, we demonstrate a positive correlation between the PHEO-to-liver SUV_{max} ratio and the mean number of secretory granule fusion events of the corresponding PHEO cells revealed by amperometric spikes ($p = 0.01$). **Conclusion:** While the ^{18}F -FDOPA PET/CT MB of PHEO strongly correlates with the concentration of MN, amperometric recordings suggest that ^{18}F -FDOPA uptake could be enhanced by overactivity of catecholamine exocytosis.

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S. Moog and S. Houy contributed equally as co-first authors, and S. Gasman and T. Cuny as co-last authors, to this work.

Introduction

Pheochromocytomas (PHEO) define tumors arising from secreting adrenomedullary chromaffin cells that commonly produce one or more catecholamines whose degradation results in the release of metanephrine (MN) and normetanephrine (NM) in plasma. Hypersecretion of catecholamines can cause sustained or paroxysmal elevations in blood pressure, headaches, episodic profuse sweating, and palpitations and can ultimately be the cause of severe cardiovascular morbidity [1, 2]. Currently, nearly 40% of PHEO encountered in the clinic can be associated with a syndrome of predisposition in which the tumors are likely recurrent, numerous, and/or metastatic [3–5].

Besides hormonal evaluation, radiological and nuclear medical imaging procedures are required for the diagnosis of PHEO, including the search for metastasis. ^{18}F -FDOPA positron emission tomography/computed tomography (^{18}F -FDOPA PET/CT) has both high sensitivity and high specificity for the characterization of these tumors, especially in cases of small hereditary PHEO [6, 7]. The principle of ^{18}F -FDOPA PET/CT is based on the property of neuroendocrine tumor cells to take up L-DOPA via the neutral amino acid transporter system L (LAT1 or LAT2), to decarboxylate it into dopamine thanks to amino acid decarboxylase, and to eventually store amino acids (such as DOPA) and their biogenic amines in vesicles [8]. A recent study suggests a positive correlation between the intensity of ^{18}F -FDOPA uptake in patients with PHEO and their respective level of urinary MN [9]. However, the relation between the ^{18}F -FDOPA PET/CT uptake parameters and the cellular pattern of catecholamine secretion in the corresponding tumors may need further clarification and new insights. A suitable technique for investigating this issue is carbon fiber amperometry, allowing the real-time measurement of catecholamine secretion at the level of a single cell [10].

Further information focusing on the potential correlation between ^{18}F -FDOPA uptake and tumor cell secretion could be of a great interest to optimize both medical and surgical approaches to treatment of these potentially life-threatening tumors.

The aim of our study was therefore (1) to evaluate the relation between ^{18}F -FDOPA PET/CT uptake and the secretion of catecholamines in patients with histologically confirmed PHEO and (2) to compare, in a subgroup of PHEO cases, the characteristics of ^{18}F -FDOPA PET/CT uptake with the profiles of tumor cell secretion recorded *in vitro* by carbon fiber amperometry.

Subjects and Methods

Patient Population

This retrospective single-center study enrolled 39 patients with histologically confirmed PHEO operated between January 2012 and June 2017 at the Department of Endocrine Surgery. Patients with parasympathetic paragangliomas were excluded from the study, because they usually do not produce catecholamines. None of the patients had a metastatic PHEO at the time of the study.

All patients underwent ^{18}F -FDOPA PET/CT at the time of initial diagnosis and a complete endocrinological evaluation before surgery, including measurement of plasma free MN and NM (nmol/L) and/or urinary MN and NM (nmol/24 h) by high-performance liquid chromatography. Chromogranin A (CGA) levels were assessed by radioimmunoassay (CgA-RIA CT; CIS Bio International, Gif-sur-Yvette, France). The upper limits of normal (ULN) of free MN and NM were 4.05 and 9.8 nmol/L in plasma and 1,625 and 2,620 nmol/24 h in urine, respectively. The upper reference limit for CGA was 100 mg/L. For the sake of consistency, we present the plasma free MN and NM as well as the urinary MN and NM and the CGA measurements as ratios normalized by the ULN. MN and NM ratios in plasma and urine were used to determine the catecholamine secretion phenotype of the PHEO, as previously described by Eisenhofer et al. [11]: in the adrenergic phenotype, the MN content exceeds 10% of the combined MN and NM content, while in the noradrenergic phenotype, the tumors present an increase in MN content which remains below 10% of the combined MN and NM content. A subset of the tumors was defined as poorly secreting if the urinary MN and NM levels were below 2 ULN.

When available, genetic assessments for germline mutations in *SDHB/D*, *VHL*, and *RET* were made. Histopathological evaluation was ultimately considered as the gold standard for the diagnosis of PHEO, and it included the tumor size, the proliferation index assessed by Ki-67 labeling (using the MIB-1 antibody), and the PASS (Pheochromocytoma of the Adrenal Gland Scaled Score) system as previously described by Thompson [12].

^{18}F -FDOPA PET/CT Acquisition Protocol

Images were obtained using a Biograph 6 true point PET/CT scanner (Siemens Medical Solutions, Knoxville, TN, USA). Image acquisition was started 66 ± 14 min after the direct intravenous injection of 300 ± 80 MBq (4 MBq/kg) of ^{18}F -FDOPA. Neither was carbidopa used for pretreatment nor was fasting required before image acquisition.

The heterogeneity of a lesion was defined during the CT scan procedure by the presence of hemorrhagic and/or necrotic tissue.

Image Analysis and Quantification of ^{18}F -FDOPA Uptake

Quantitative parameters of ^{18}F -FDOPA uptake were obtained using IntelliSpace Portal 6.4 ISP (Philips), and they included the different standardized uptake values (SUV; i.e., SUV_{max} , SUV_{mean} , lesion-to-liver SUV_{max} , and lesion-to-liver SUV_{mean}). All ^{18}F -FDOPA PET/CT scans were interpreted by the same physician. All of these values were obtained from the volume of PHEO, which was encompassed by an axial slice-by-slice approach. The SUV is the ratio between the image-derived radioactivity concentration and the whole-body concentration of the injected radioactivity; it was calculated as follows: $\text{SUV} = \text{radioactivity concentration in a region of interest (kBq/mL)} / (\text{patient weight [g]} / \text{injected dose})$.

[kBq]). The highest SUV in all the tumor volume was defined as the SUV_{max}, whereas the average of the SUV was defined as the SUV_{mean}. A normal liver region was defined by drawing a region of interest to derive tumor-to-background ratios (PHEO-to-liver SUV) in order to facilitate comparisons between patients.

The metabolic burden (MB) was determined to serve as an image-derived index of overall tumor activity per patient, as previously described [13], and was calculated for each adrenal lesion as follows: MB = SUV_{mean} × tumor volume. If the patient had bilateral PHEO, the whole-body MB was calculated as the sum of the MB of each lesion.

Primary Culture of Human PHEO

After resection, the PHEO was cut longitudinally in two parts, and a piece of the tumor zone was dissected and placed in the operating room in CMF HBSS (Hank's Balanced Salt Solution without Ca²⁺ and Mg²⁺: 0.3 mM Na₂HPO₄, 4.1 mM NaHCO₃, 0.4 mM KH₂PO₄, 138 mM NaCl, 5.3 mM KCl, 5.5 mM glucose, 1.2 mM CaCl₂, 0.5 mM MgCl₂, and 0.4 mM MgSO₄) supplemented with 0.2% FBS (fetal bovine serum) and 1% penicillin/streptomycin. The tumor was cut into small pieces (1 mm³) before centrifugation at 250 g for 5 min in complete RPMI (RPMI, 15% FBS, and 1% penicillin/streptomycin).

A pellet was resuspended in complete RPMI and settled during 15–30 min at room temperature in order to eliminate blood cells and fat with the supernatant. This step was repeated twice. The tumor pieces were washed in CMF HBSS, centrifugated at 250 g for 5 min, then dissociated with 1.5 mg/mL of collagenase B (Roche) with 1 mg/mL of dispase II (Gibco) in HBSS during 45 min at 37 °C under moderate agitation. After sedimentation, the supernatant was recovered (fraction 1) and the pellet crushed in CMF HBSS. After renewed sedimentation, the supernatant was recovered again (fraction 2). Both fractions were centrifugated for 5 min at 800 g at room temperature and resuspended into 3 mL of complete RPMI. Three hundred microliters of suspended cells were plated on polylysine-coated cover glass (MatTek). The cells were then incubated at 37 °C in a water-saturated atmosphere with 5% CO₂, and complete RPMI was added the following day.

Carbon Fiber Amperometry

Only patients with sporadic PHEO and homogeneous lesions on the CT scan were included. Two days after culture, chromaffin cells originating from PHEO were washed with Locke's solution (140 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 0.01 mM EDTA, 15 mM HEPES, and 11 mM glucose; pH 7.5) and processed for catecholamine release measurements by amperometry as previously described [14].

Briefly, a carbon fiber electrode of 5 μm diameter (ALA Scientific Instruments) was held at a potential of +650 mV compared with the reference electrode (Ag/AgCl) and approached close to the cells. Secretion of catecholamines was induced by a 10-s pressure ejection of 100 mM potassium (K⁺) solution from a micropipette positioned 10 μm from the cell and recorded over 60 s. The amperometric recordings were performed with an AMU130 amplifier (Radiometer Analytical), calibrated at 5 kHz, and digitally low-pass filtered at 1 kHz.

An analysis of the amperometric recordings was performed as previously described with a macro (laboratory of Dr. R. Borges; <http://webpages.ull.es/users/rborges/>) written for Igor software (WaveMetrics), allowing automatic spike detection and extraction

Table 1. Clinical, biochemical, and functional characteristics of the 39 patients with PHEO

<i>Clinical characteristics (n = 39)</i>	
Age, years	53±15
Female	22 (56%)
Presentation	
Incidentaloma	13 (22%)
Hypertension	24 (60%)
Symptoms related to abdominal mass	11 (27%)
<i>Paraclinical characteristics (n = 39)</i>	
Germline mutations	
RET	12/35
VHL	3
NF1	1
SDHB	3
SDHD	2
SDHD	3
Biochemical analysis	
Adrenergic/noradrenergic phenotype	
Total urinary MN and NM, ULN ratio (n = 34)	26/13
Urinary MN, ULN ratio (n = 37)	6.3±6.6
Urinary NM, ULN ratio (n = 37)	7.5±11.7
Plasma free MN, ULN ratio (n = 22)	6.8±6.8
Plasma free NM, ULN ratio (n = 23)	5.5±8.1
CGA, mg/L (n = 30)	5.6±6.1
CGA, mg/L (n = 30)	345±443
¹⁸ F-FDOPA PET/CT uptake parameters	
SUV _{mean}	8.9±5.0
SUV _{max}	10.4±6.1
Metabolic burden, cm ³	260±375
Histology	
Size, cm	4.5±2.6
Bilateral tumor	2 (5%)
Metastatic tumor	0 (0%)
PASS	1 (0–4)
Ki-67 index	2 (0–6)

Values are presented as means ± SD/(range) or n (%). PHEO, pheochromocytoma; VHL, von Hippel-Lindau tumor suppressor; NF1, neurofibromatosis type 1; SDHx, succinate dehydrogenase; MN, metanephrine; NM, normetanephrine; CGA, chromogranin A; ULN, upper limit of normal; SUV, standardized uptake value; PASS, Pheochromocytoma of the Adrenal Gland Scaled Score.

of spike parameters [15]. The spike parameter analysis was restricted to spikes with amplitudes higher than 5 pA. The number of amperometric spikes was counted as the total number of spikes with an amplitude greater than 5 pA within 60 s. We investigated the total number of spikes per cell corresponding to the number of exocytotic events, the quantal size of individual spikes (Q) corresponding to the spike area above the baseline, which is proportional to the amount of amines per granule, and the spike amplitude (I_{max}), which corresponds to the maximal flow of catecholamines released per granule.

Statistical Analysis

For statistical analysis, GraphPad Prism[®] 7.0 (GraphPad Software, San Diego, CA, USA) was used. The data are reported as

means \pm SD or SEM. Comparative statistical evaluations between groups (biological phenotype clusters or genotype clusters) were accomplished with a Mann-Whitney test followed by Tukey's test for multiple post hoc comparisons. For correlations between parameters of ^{18}F -FDOPA uptake and biological measurements or amperometric experiments, Spearman's rank order was performed. A p value <0.05 was considered to be significant for all tests.

Results

Overall Patient Characteristics

This study included 39 patients (17 males and 22 females; mean age 53 ± 15 years) who had 41 histologically proven PHEO (2 patients had bilateral tumors). Hypertension was found in 24 patients (60%) at the time of diagnosis. Incidentaloma was the mode of presentation in 13 patients (22%), a proportion higher than the 5% reported in the literature [16]. Biochemically, an adrenergic secretion phenotype, according to Eisenhofer's criteria, was found in 26 patients (65%) who presented a mean urinary MN level 11-fold above the ULN. The remaining thirteen patients had a biochemical phenotype in line with an oversecretion of norepinephrine, as their mean level of urinary NM was 6-fold upper the normal limit value (Table 1). In total, 6 patients (15%) were considered as having poorly secreting tumors.

A total of 35 patients were screened for germline mutations, and amongst them, 12 (34%) carried mutations as follows: *SDHD* ($n = 3$), *SDHB* ($n = 2$), *NF1* ($n = 3$), *VHL* ($n = 1$), and *RET* ($n = 3$). Half of them had already been surgically treated for other contralateral PHEO or parasympathetic paragangliomas.

Finally, the median histopathological PASS was 1 (range 0–4), with a median Ki-67 index of 2 (range 0–6) and a mean tumor size of 4.5 ± 2.6 cm (Table 1). While there was no statistically significant difference in tumor size between the adrenergic and noradrenergic phenotypic subgroups, the PHEO were of a significantly smaller size in the 12 patients with germline mutations, including the 5 *SDHB/D* mutations, than in their nonmutated counterparts (3.1 ± 2.3 vs. 6.1 ± 2.9 cm, respectively; $p = 0.005$).

^{18}F -FDOPA PET/CT and Hormonal Secretion Data

^{18}F -FDOPA PET/CT data were available for all patients. The mean SUV_{max} and SUV_{mean} of the lesions were 10.4 ± 6.1 and 5 ± 3.3 , respectively. The mean tumor volume was 69 ± 101 cm^3 and the mean MB was 260 ± 375 cm^3 . Evaluating the usefulness of ^{18}F -FDOPA PET/CT in

the determination of catecholamine secretion, we found a statistically significant positive correlation between the tumor MB and the corresponding level of 24-h urinary NM ($r = 0.64$, $p < 0.0001$, $n = 36$; Fig. 1a), MN ($r = 0.49$, $p = 0.002$, $n = 35$; Fig. 1b), and combined MN and NM ($r = 0.75$, $p < 0.0001$, $n = 33$; Fig. 1c). Similarly, a positive correlation was observed between the tumor MB and the concentrations of plasma free NM ($r = 0.55$, $p = 0.006$, $n = 23$; Fig. 1d) and CGA ($r = 0.61$, $p = 0.0005$, $n = 28$; Fig. 1e) but not with plasma MN ($r = 0.11$, $p = 0.6$, $n = 22$; data not shown).

In contrast, there was no correlation between the SUV characteristics of the PHEO (SUV_{mean} or SUV_{max}) and the corresponding concentrations of MN or NM either in plasma or in the 24-h urinary sample (data not shown), except for the plasma levels of CGA, for which a positive correlation was observed with both the SUV_{mean} and the SUV_{max} of the PHEO ($r = 0.41$, $p = 0.02$, and $r = 0.55$, $p = 0.0019$, respectively; data not shown).

In addition, the 6 poorly secreting PHEO showed a trend for both a lower MB ($p = 0.06$) and a lower size of the tumors (3.35 ± 1.2 cm vs. 5.1 ± 0.4 cm; $p = 0.07$) compared to the secreting PHEO. A significantly lower MB was observed in the 12 mutated PHEO as compared to the sporadic PHEO (87 ± 109 vs. 385 ± 494 cm^3 ; $p = 0.008$), mainly due to the fact that the mutated PHEO had a smaller size than the nonmutated tumors (see earlier). Of note, the MB of the mutated PHEO, even when they were smaller, positively correlated with urinary levels of MN ($r = 0.69$, $p = 0.01$, $n = 11$; data not shown), NM ($r = 0.64$, $p = 0.03$, $n = 11$; data not shown), and combined MN and NM ($r = 0.86$, $p = 0.0012$, $n = 10$; Fig. 1f). In the subgroup of PHEO with *SDHB* and *SDHD* mutations (MB 24 ± 23 cm^3), we did not find any correlation with urinary levels of catecholamines ($r = 0.4$, $p = 0.75$; data not shown).

At the histopathological level, the PASS and/or the Ki-67 proliferation index of the tumor did not correlate one way or another with its corresponding ^{18}F -FDOPA PET/CT uptake parameters (data not shown).

In summary, we found a positive correlation between the MB of the PHEO as calculated by ^{18}F -FDOPA PET/CT and their respective circulating (plasma) or excreted (urinary) levels of MN and/or NM. We then wondered if, besides the concentration of MN, the pattern of catecholamine secretion as measured by amperometry could also influence the ^{18}F -FDOPA PET/CT uptake by the tumor.

Amperometry Analysis

Among our samples, 5 freshly resected PHEO originating from 5 distinct patients (2 women and 3 men; all

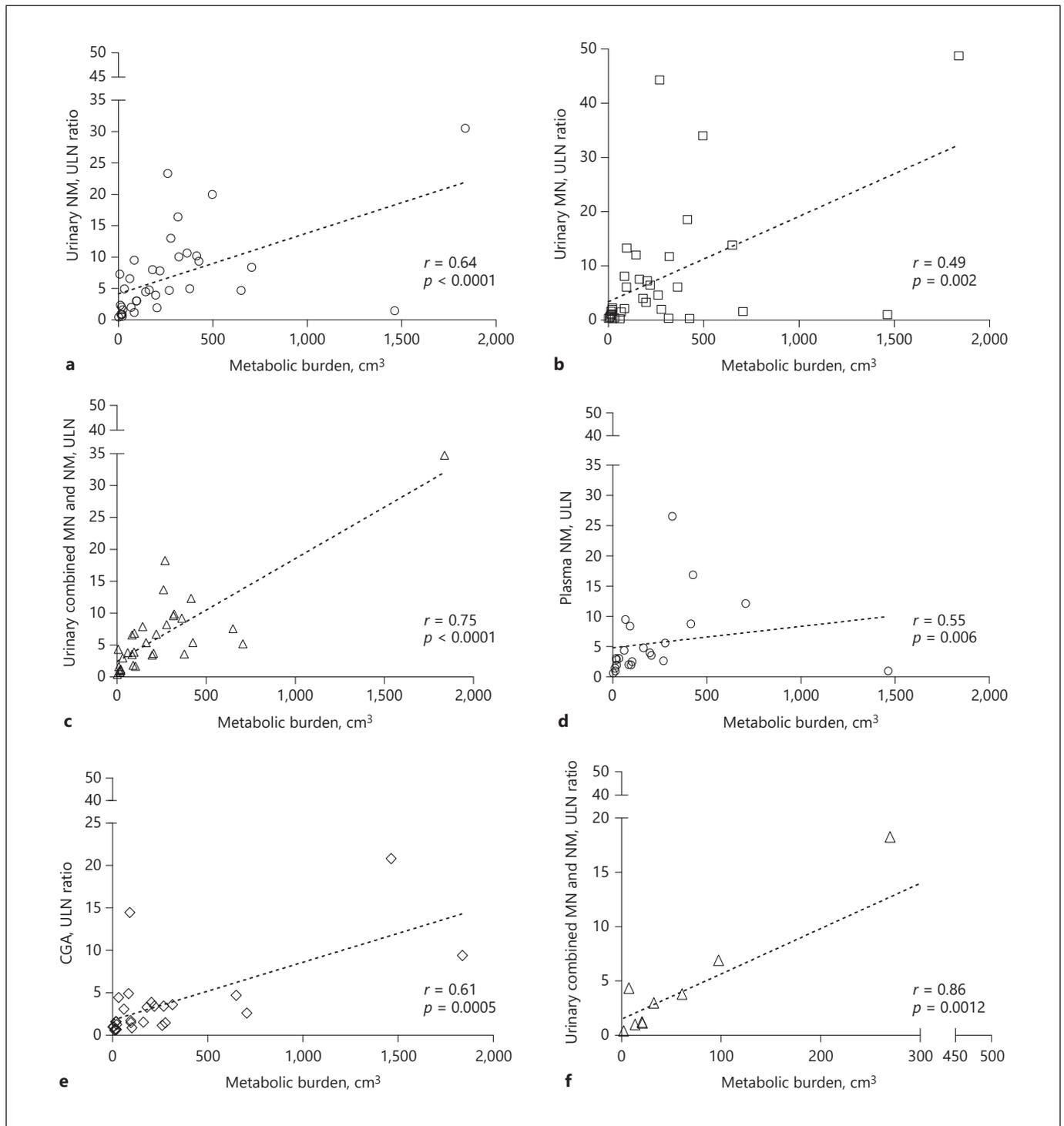
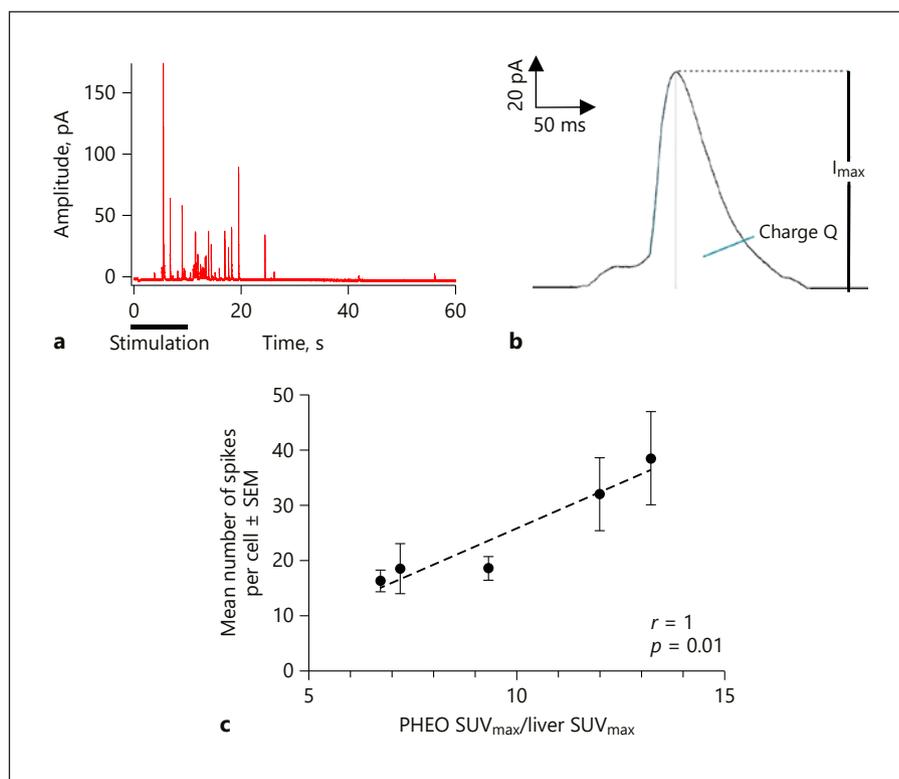


Fig. 1. Correlation between the ¹⁸F-FDOPA PET/CT metabolic burden of the pheochromocytomas and the respective upper limit of normal (ULN) ratios of 24-h urinary normetanephrine (NM) (a), 24-h urinary metanephrine (MN) (b), 24-h urinary combined MN and NM (c), plasma free NM (d), plasma chromogranin A (CGA) (e), and 24-h urinary combined MN and NM in the subgroup of mutated pheochromocytomas ($n = 11$) (f). For correlation, Spearman's rank order was performed.

Fig. 2. **a** Amperometric recording of catecholamine release after stimulation of one chromaffin cell. **b** Schematic representation of one amperometric spike. Each spike represents a single granule fusion event, with the surface area or quantal size (Q) being proportional to the amount of catecholamine released per event, and the spike height value (I_{\max}) displaying the maximal flux of catecholamines. **c** Correlation between the mean number of spikes recorded by amperometry in primary culture of 5 pheochromocytomas (PHEO) and their corresponding lesion-to-liver maximum standardized uptake value (SUV_{\max}) ratio. For correlation, Spearman's rank order was performed.



PHEO sporadic, 3 of the adrenergic and 2 of the noradrenergic phenotype) were placed in primary culture in order to perform real-time single-cell catecholamine secretion measurements using carbon fiber amperometry [10]. Tumor chromaffin cells were stimulated with a depolarizing concentration of K^+ for 10 s and amperometric spikes were measured over a period of 60 s. A representative amperometric trace recorded from a chromaffin cell cultured from the PHEO of patient 2 is illustrated in Figure 2a. Each spike represents a single granule fusion event, with the surface area or quantal size (Q) being proportional to the amount of catecholamines released per event, and the spike amplitude value (I_{\max}) displaying the maximal flux of catecholamines (Fig. 2b). Table 2 summarizes the ^{18}F -FDOPA PET/CT and amperometric parameters for this subgroup of PHEO.

The mean number of spikes detected per cell was 24.7 ± 4.4 , the mean area under the spike trace (Q) was 0.83 ± 0.12 pC, and the mean I_{\max} was 16 ± 2.0 pA. No correlation was found between either the SUV or the MB of the tumor and the corresponding quantal size Q or the spike height value I_{\max} (data not shown), meaning that the amount of catecholamine stored per secretory granule does not seem to influence the ^{18}F -DOPA uptake of the tumor.

It is noteworthy that we observed for the first time a strictly positive correlation between the lesion SUV_{\max} /liver SUV_{\max} ratio and the number of spikes per cell obtained from the corresponding tumor ($r = 1$, $p = 0.01$; Fig. 2c), which suggests that the amount of uptake (as reflected in the SUV_{\max}) is related to the total number of exocytotic events.

Discussion

Nuclear medical imaging modalities play a pivotal role in the management of patients with adrenal lesions, especially PHEO [6]. Over the past years, PET/CT examinations have marked a step forward regarding localization, relapse, and assessment of the aggressiveness of the disease. Recent studies also suggest that PET/CT may, in certain circumstances, yield information about the functional status of an adrenal lesion, especially in case of cortisol-secreting lesions [17].

In this study, in a series of 39 operated PHEO we found a positive correlation between ^{18}F -FDOPA MB and the corresponding urinary and/or plasma concentrations of MN and NM. While only a few studies have been pub-

Table 2. Clinical, functional, and amperometric characteristics of 5 primary cultures of human pheochromocytomas

Patient No.	Sex	Tumor size, mm	Secretion phenotype	Lesion SUV _{max} /liver SUV _{max}	Metabolic burden, cm ³	Number of cells analyzed by amperometry	Mean number of spikes per cell ± SEM	Mean charge Q ± SEM, pC	Mean amplitude I _{max} ± SEM, pA
1	F	80	AD	7.2	650	14	18.4±4.5	1.27±0.10	13.7±0.80
2	M	37	AD	12	84	19	32±6.6	0.90±0.10	21.3±2.16
3	F	50	AD	9.3	220	23	18.6±2.2	0.68±0.05	11.3±0.81
4	M	44	NAD	6.7	378	21	16.3±2.0	0.81±0.10	20.2±2.71
5	M	40	NAD	13.2	278	6	38.5±8.5	0.51±0.12	13.7±2.90
Mean ± SEM		50±7.7		9.6±1.3	322±94	16.6±3.0	24.7±4.4	0.83±0.12	16±2.0

SUV, standardized uptake value; AD, adrenergic phenotype; NAD, noradrenergic phenotype.

lished on the topic, some similar observations have been made by others in the past [9, 18, 19]. However, there is still no consensus concerning the best predictive ¹⁸F-FDOPA PET/CT uptake parameter to be used in clinical practice. Like ours, the recent study published by Amodru et al. [9] was a retrospective and single-center trial. In their study, 56 patients with a median age of 47.5 years were included. There were fewer patients with a genetic mutational background ($n = 11$; 19.6%), including 5 *RET*, 2 *SDHB*, 2 *SDHD*, 1 *NFI*, and 1 *VHL* mutation. The uptake parameters assessed during the ¹⁸F-FDOPA examination included tumor SUV_{max}, tumor SUV_{mean}, MTV 42% (defined by the region enclosed by a 42% isocontour around the maximal PET voxel), and total lesion uptake, which corresponded to tumor SUV_{mean} × MTV 42%. In that study, the tumor SUV_{max}, SUV_{mean}, and tumor SUV_{max}-to-liver SUV_{max} ratio showed a statistically significant correlation with urinary MN levels. Interestingly, significant positive correlations were observed between the value of urinary MN and MTV 42% and total lesion uptake, respectively, a result close to the one observed in our study with the MB.

The study by Amodru et al. [9] showed that the SUV_{max} of PHEO correlates with the concentration of circulating catecholamines, whereas Imani et al. [19] did not find any statistically significant correlation between the SUV_{max} of PHEO and the corresponding biochemical markers. This discrepancy could be due to the SUV_{max} parameter itself, which is subject to many sources of variability that are not controlled or even taken into account in most reported studies and which reflects a single-pixel value [20, 21]. The synthesis of catecholamines and their subsequent secretion in PHEO, albeit heterogeneous, are likely to occur in all parts of the tumor, so that whether the SUV_{max} is a reliable tool for predicting the capacity of the tumor to secrete catecholamines is questionable. Un-

til now, the SUV_{max} has mostly been used to predict the malignancy or proliferative activity [17, 22, 23] rather than the secretion status of different types of tumors – including adrenal cortical tumors, in which it strongly correlates with the definitive histopathological Weiss score system [24].

Our study shows that the MB of PHEO, which takes into consideration both the mean of the SUV and the volume of the lesion, estimates the concentration of circulating catecholamines, since the “real” measurement of catecholamine secretion by the tumor would require a sophisticated approach including tumor sampling. Two other previous studies also identified a positive correlation between biochemical parameters of medullary thyroid carcinoma and carcinoid tumor and the whole MB of the tumor as calculated during ¹⁸F-FDOPA PET/CT [25, 26].

Using the MB as a predictor of catecholamine concentration is subject to the fact that the size of a PHEO positively correlates with the concentration of NM and MN, as suggested in a previous study [27]. As such, a trend for a lower MB was observed in our subgroup of poorly secreting PHEO (defined by an intermediate secretion status below 2 ULN) compared to their secreting counterparts. While conducting this study, we wondered if similar concentrations of MN and NM would be observed in a PHEO alternatively secreting high amounts of catecholamines followed by periods of quiescence (i.e., paroxysmal secretion) and in a PHEO which would permanently release moderate levels of catecholamines. While, biochemically, it could lead to similar concentrations, a strong difference remains in terms of the pattern of secretion, and whether the uptake of ¹⁸F-FDOPA could be influenced by this pattern of secretion is currently unknown. This hypothesis prompted us to compare, in the second part of this work, the ¹⁸F-FDOPA uptake param-

eters of 5 PHEO with their respective capacity of secretion reflected in the number of exocytotic events obtained after stimulation by amperometric recordings. To the best of our knowledge, we here show for the first time in a translational approach that a positive correlation exists between the PHEO-to-liver SUV_{max} ratio and the mean number of spikes per cell recorded during carbon fiber amperometry. Our results thus suggest that the more important exocytic activity the PHEO has, the more uptake of ^{18}F -FDOPA occurs, leading to high SUV_{max} values.

In contrast, no correlation was observed between any of the amperometric parameters of a PHEO and its MB, and several hypotheses may subsequently be discussed. For instance, the MB reflects the metabolic activity of the lesion in its entirety, which means that the result obtained is the synthesis of poorly as well as highly secreting PHEO cells. However, amperometric experiments are conducted on a single cell, being either a poorly, an intermediately, or a highly secreting cell. This could also be the reason why a positive correlation is rather observed with the SUV_{max} of a PHEO, since this parameter assesses a highly active zone of the tumor where cells take up considerable amounts of ^{18}F -FDOPA and, therefore, are likely all to be highly secreting. Following this point, it is therefore not surprising to observe that the MB, which represents the whole uptake of a PHEO at the metabolic imaging level, positively correlates with the level of urinary/plasmatic catecholamines, which reflect the secretory activity of the entire PHEO at the biological level.

An unresolved question that was raised with the amperometric results of our study is the peculiar aspect of head and neck paragangliomas, which are tumors with usually high uptake of ^{18}F -FDOPA but normal concentrations of plasma MN. Most of human paragangliomas, whatever their primary location, express high levels of LAT, the neutral amino acid transporter system L involved in the uptake of DOPA [28], which may explain their propensity to take up high levels of the radiotracer. However, as compared to PHEO, paragangliomas have a lower expression of the genes involved in catecholamine synthesis (i.e., tyrosine hydroxylase and/or phenylethanolamine N-methyltransferase) [29, 30]. As such, a hypothesis could be that certain paragangliomas have the molecular equipment required for DOPA uptake, albeit without the capacity to insure catecholamine production and secretion. In order to clarify these questions, amperometric experiments will have to be conducted on paragangliomas.

Finally, our study has obvious inherent limitations due to its retrospective nature. In this respect, we only includ-

ed patients with well-characterized biological data and complete ^{18}F -FDOPA PET/CT reports. Because we included surgically treated patients, meaning that a majority of these patients should have been diagnosed with symptoms of catecholamine hypersecretion, correlations might be slightly overestimated. However, an unexpectedly high prevalence of incidental PHEO was noted in our series – at least higher than the one reported in the literature. Also, over the study period, ^{18}F -FDOPA PET/CT acquisition has not been performed according to a strict protocol, as is the case in prospective studies, and could therefore be the source of reproducibility bias. To avoid excessive variability between the data, all of our ^{18}F -FDOPA PET/CT scans were interpreted by the same physician. Finally, there were only a small number of mutated PHEO and a limited representation of specific mutations due to their relative rarity in our series, which also constitutes a limitation of this study. As such, only sporadic PHEO were used in the amperometric analysis, because mutated PHEO do not have the same pattern of ^{18}F -FDOPA uptake [7]. Nevertheless, other experiments will have to be performed on this subgroup of patients to confirm or disprove these data.

In conclusion, PHEO are life-threatening tumors which are the source of significant morbidity in clinical practice [31]. Rather than assessing the total amount of catecholamines, it appears critical to improve our understanding of the mechanisms involved in the pattern of secretion displayed by these tumors, as PHEO-induced mortality mainly occurs during an adrenergic storm, when the tumors suddenly release considerable amounts of catecholamines [32]. While the MB as provided by ^{18}F -FDOPA PET/CT correlates with the concentration of catecholamines, further investigation is needed to specify whether the SUV_{max} may serve as a reliable tool to predict the secretion pattern of the tumor, as suggested by amperometric experiments.

Statement of Ethics

The present study was approved by the Ethics Committee of the University Hospital of Nancy (Nancy, France), and informed consent was obtained from each patient.

Disclosure Statement

The authors have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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