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Original Research

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Plasma metanephrines responses to adreno-sympathetic stress

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ABSTRACT

Background: Plasma metanephrines (MNs) measurement is a highly sensitive method for the diagnosis of catecholamine producing tumors. Pre-analytical factors like non-specific adreno-sympathetic stimulations may alter the specificity of the test. **Methods:** Free catecholamines and MNs were determined in plasma after three adreno-sympathetic stimulations: Postural change from supine to standing the position, insulin-induced hypoglycemia test and a cold pressure test. **Results:** Sympatho-neuronal stimulation such as orthostatic stimulation and cold exposure mainly stimulated the release of norepinephrine (NE) (+61% and +34%, respectively) and in a weaker way of adrenaline (+28% and +16%, respectively). The resulting rise in normetanephrine (NMN) and MN is much more attenuated after orthostatic change (+30% and +22%, respectively) and cold exposure (+19% without change for MN). Insulin-induced hypoglycemia elicited a massive release of epinephrine from the adrenal medulla (+1587%) and to a lesser extent that of NE from sympathetic neurons (+134%). Plasma NMN and MN peaked 15 min later, and peaks were more attenuated (+375% and +58%, respectively). Plasma concentrations exceeded the threshold of the paragangliome detection test in 15% of patients for NMN after orthostatic stimulation and 76% of patients for MN after hypoglycemic stress. **Conclusion:** A moderate sympatho-excitatory stimulation such as exposure to cold has little influence on plasma MNs. In contrast, postural changes and metabolic stresses can lead to falsely positive interpretations of the detection test confirming the requirement of blood sampling after at least 30 min of rest in the supine position.

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INTRODUCTION

The diagnosis of catecholamine producing tumors, pheochromocytoma and paraganglioma depends on the demonstration of elevated production of catecholamines and their 3-O-methylated metabolites. Catecholamines are secreted in an intermittent fashion and between these episodes catecholamines levels may be normal [1]. Moreover, catecholamines are intensively metabolized within the tumors to their 3-O-methylated metabolites [2]. According to the recommendations from the First International symposium on pheochromocytoma, the measurement of plasma levels of free metanephrines (MNs) represents a more effective mean to diagnose paraganglioma [3].

Recently, we demonstrated the usefulness of a radioimmunoassay for the diagnosis of catecholamines producing tumors in a population suspected of pheochromocytoma. Using an analysis of receiver-operating characteristic curves, we established cut-off values for plasma normetanephrine (NMN) (100 pg/ml) and MN (70 pg/ml) leading to high sensitivity (97% and 61%, respectively) and specificity (96% and 97%, respectively) [4]. Nevertheless, it is sometimes difficulty in distinguishing false-positive from true-positive results. The interpretation of biochemical results may be affected by pre-analytical factors such as medications or dietary and blood sampling was recommended after an overnight fast [5,6]. Clinical pathologies such as hypertension, cardiac failure, renal failure, or monoamine oxidase deficiency activated sympathetic outflow with a smaller proportional increase in plasma MNs than catecholamines [7]. Similarly, infusion of ^{3}H-labeled catecholamines increased plasma concentrations of free MNs by <10% of the rises in precursor amines [8,9]. Physiological stimulations of the sympathetic system, such as exercise, posture modification, mental or metabolic stress and cold exposure diversely enhance the production of catecholamines [10,11]. Even if the rise in free plasma MNs is attenuated compared to that of catecholamines, these stimuli may alter the diagnostic accuracy of plasma free MNs.

In the present study, the concentrations versus time profiles of MNs were compared to those of their precursors during three physiological stresses. The impact of these nonspecific stimulations on the performances of the biochemical test was discussed.

METHODS

Sympathetic Stimulations Tests

Catecholamine and MNs concentrations were determined in plasma during three sympathetic stimulation tests. The study was carried out in accordance with ethical guidelines for experiments involving humans. The subjects gave informed consent to participate.

Postural Stimulation

The effect of postural change was evaluated in 70 patients presenting for the exploration of hypertension and/or incidentaloma. Their age (mean ± standard deviations [SD]) was 59 ± 16 years with a sex ratio (M:F - 40:30). They were allowed to rest in the supine position for at least 30 min. Blood samples were withdrawn through an antecubital cannula during supine rest and after at least 30 min in standing the position and moderate activity.

Insulin Tolerance Test

Plasma free catecholamines and MNs were measured in 20 supine patients undergoing an insulin-induced hypoglycemia test for the exploration of the anterior pituitary function. Their age (mean ± SD) was 38 ± 15 years with a sex ratio (M:F - 12:8). Hypoglycemia was induced by an intravenous bolus of insulin (Actrapid®) at the dose of 0.1 IU/kg body weight [11]. Blood samples were obtained before (−15 min and just before) and 15, 30, 45, 60, 90, and 120 min after insulin injection. Glycemia, plasma catecholamines and MNs were determined.

Cold Pressor Test

Cold pressor tests were performed in 6 healthy volunteers aged of 45 ± 10 years with a sex ratio (M:F - 3:3) in the supine position. They immersed the right hand up to the wrist level into ice-cold water (0-4°C) for 3 min. Blood samples were withdrawn 5 min before and 5, 10, 15, 30, 45, and 60 min after the onset of the cold pressor test as previously described [10].

Analytical Methods

Blood samples were collected into EDTA-containing vacutainer tubes. All blood samples were immediately centrifuged at 800 g for 15 min at 4°C. Plasma samples were stored at −20°C until analysis within 1 week after collection. Concentrations of catecholamines in plasma were quantified by liquid chromatography with electrochemical detection after extraction on alumina (chromsystems instruments and chemicals [München, Germany]). The recoveries were between 69 and 81% for norepinephrine (NE) and epinephrine. The detection limit was 20 pg/ml. The inter-assay coefficients of variation were 9.5% for NE (251 ± 24 pg/ml) and 12% for epinephrine (42 ± 5.1 pg/ml) [4]. Free plasma MNs were determined using a radioimmunoassay commercial kit manufactured (IBL, GmbH, Hamburg, Germany) [4,12]. Briefly, 500 μL of standards, controls, and plasma samples were purified through C18 extraction columns. After elution, MNs were acylated using a reagent NHS-Biotin. After evaporation in an evaporator centrifuge, the remaining acylated MNs were determined using a competitive radioimmunoassay. The recoveries for NMN and MN were 93% and 96%, respectively. The sensitivity limits were 10 pg/ml and 4 pg/ml for NMN and MN, respectively. This method shows a satisfactory precision with intra- and inter-assay below 15% in the normal range and below 10% in the pathological range of concentrations.

Statistical Analysis

The Shapiro–Wilk normality test was used to evaluate the data distribution. Non-normally distributed data were compared using Mann–Whitney test. Hormonal changes were analyzed using repeated measures analysis of variance and compared two by two using Bonferroni multiple comparison test. The level of significance was set at $P < 0.05$.

Cut-off values were calculated from plasma free MNs concentrations determined in supine or standing position. These upper reference limits were defined as the 97.5^{th} percentile calculated from the logarithmically transformed individual values from patients in supine or standing position. The 97.5^{th} percentile was obtained from the antilogarithm of the mean ± 2 SD of the transformed data. Then, these thresholds have been tested on the cohort of 533 patients (59 patients with paraganglioma and 474 patients without identifying tumors) previously described [4].

RESULTS

Postural Change

The change from supine to upright position increased both median (range) plasma NE (273 pg/ml [146-581]-458 pg/ml [205-1024], $P < 0.001$) and NMN [39 pg/ml (10-146)-51 pg/ml (16-186), $P < 0.001$] [Figure 1]. The percent rise (median [range]) in NE following the postural stress (61% [7-252]) exceeded that of NMN (30% [18-390], $P = 0.003$).

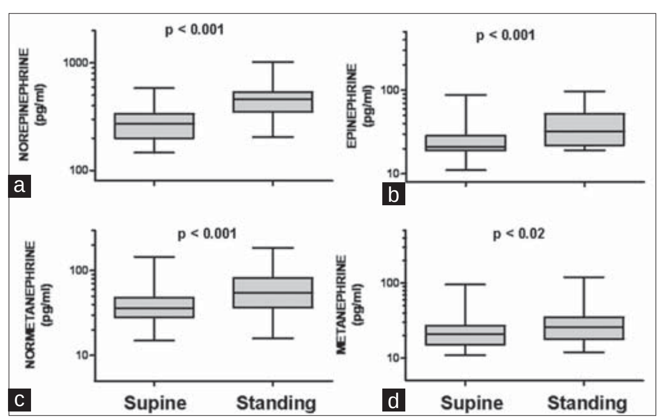

Figure 1: Median concentrations, interquartiles, and ranges of plasma free norepinephrine (a), normetanephrine (b), epinephrine (c), and metanephrine (d) in patients after orthostatic stimulation

Simultaneously, the orthostatic stress increased both epinephrine (21 pg/ml [11-97]-32 pg/ml [19-96], $P < 0.001$) and MN (21 pg/ml [11-95]-26 pg/ml [12-120], $P < 0.02$). The percent changes in epinephrine and MN plasma concentrations remained similar during orthostatic stress (28.0% [−31.0-308.0] and 22.0% [−21.0-112.0], respectively).

Cut-off values determined from the data of patients in supine position (95 and 65 pg/ml for NMN and MN, respectively) are in agreement with those previously established using receiver operating characteristic curves analysis in a population suspected of paraganglioma [13]. The cut-off values were also calculated from data in standing patients: Applying the upper limit value for NMN (160 pg/ml), the diagnostic sensitivity decreased from 97% to 86%, excluding 5 paraganglioma with borderline NMN levels. Applying the upper limit value for MN (85 pg/ml), the sensitivity of the test remained similar (59 % vs. 61 %).

Cold Pressor Test

The 3 min-cold pressor test increased both catecholamines and MNs in plasma [Table 1a]. As compared to pretest value (308 ± 70 pg/ml), plasma NE increased at 5 min (378 ± 106 pg/ml, $P < 0.001$), 10 min (393 ± 61 pg/ml, $P < 0.001$) and 15 min (357 ± 65 pg/ml, $P < 0.05$) after immersion in cold water. Compared to pretest value (52 ± 19 pg/ml), plasma NMN increased at 15 min (60 ± 20 pg/ml, $P < 0.001$) and 30 min (57 ± 22 pg/ml, $P < 0.05$) and returned to basal values at 45 and 60 min. The time to peak of NMN was later than that of NE (14 ± 2 vs. 8 ± 3 min, $P = 0.03$, respectively). Simultaneously, the cold press or test slightly increased the plasma concentrations of epinephrine at 5 and 10 min (47 ± 15 pg/ml vs. 52 ± 15 pg/ml, $P < 0.05$ and 54 ± 16 pg/ml, $P < 0.05$) without any change in MN concentrations. These variations expressed in percent changes of pre-test concentrations are illustrated in Figure 2.

Insulin Tolerance Test [Table 1b]

Insulin administration induced a 70% decrease in glycemia (4.9 ± 0.7 mmol/l-1.8 ± 0.8 mmol/l, $P < 0.001$) 30 min after insulin injection [Figure 3a]. As compared to pre-test value, NE increased from 293 ± 122 pg/ml to 530 ± 197 pg/ml ($P < 0.001$) at 30 min, 680 ± 167 pg/ml ($P < 0.001$) at 45 min and 583 ± 203 pg/ml ($P < 0.001$) at 60 min after insulin injection. As compared to pre-test values (42 ± 12 pg/ml), NMN concentrations rose at 45 min (60 ± 28 pg/ml, $P < 0.001$) and at 60 min (65 ± 22 pg/ml, $P < 0.001$). The time to peak for NMN was later (59 ± 16 min) than that for NE (45 ± 11 min, $P = 0.007$). The time-course of NE and NMN are expressed in percent changes of pretest values and illustrated in Figure 3b.

Compared to basal value (43 ± 37 pg/ml), epinephrine concentrations increased markedly at 30 min (722 ± 384 pg/ml, $P < 0.001$), at 45 min (601 ± 345 pg/ml, $P < 0.001$) and at 60 min (275 ± 351 pg/ml, $P < 0.001$) after insulin injection. MN increased from baseline value (23 ± 10 pg/ml) to 62 ± 45 pg/ml ($P < 0.005$) at 30 min, 98 ± 50 pg/ml ($P < 0.001$) at 45 min,

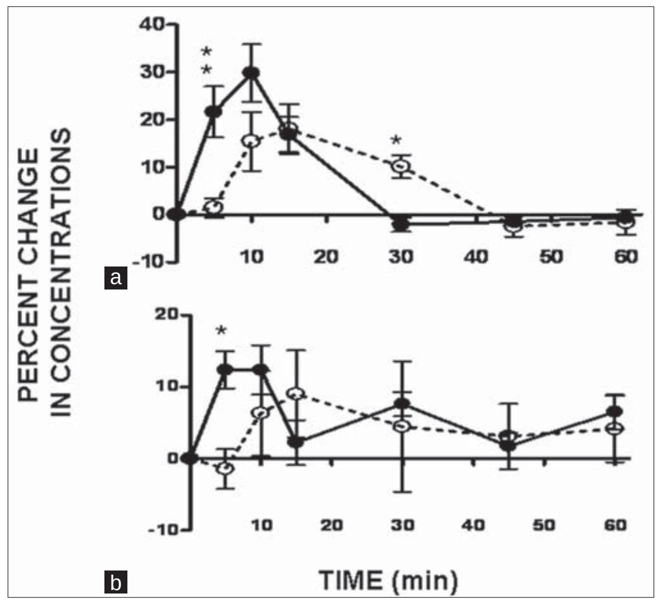

Figure 2: Changes from pre-test values in plasma catecholamines and metanephrines (MNs) (mean ± SEM) after sympathetic stimulation with cold pressor test in healthy subjects ([a] norepinephrine [continuous line] and normetanephrine [broken line], [b] epinephrine [continuous line] and MN [broken line]). $^{**}P < 0.01$ and $^{*}P < 0.05$, catecholamine versus MN

Table 1: Means±standard deviations of glycemia, catecholamines, and metanephrines concentrations determined during the cold pressor test (a) and the insulin tolerance test (b)

a: Cold pressor test							
	Pre-test	5 min	10 min	15 min	30 min	45 min	60 min
NE (pg/ml)	308±70	378±106	393±61	357±65	302±72	304±71	309±64
NMN (pg/ml)	52±19	53±21	58±15	60±20	57±22	52±21	53±17
E (pg/ml)	47±15	52±15	54±16	48±15	51±14	48±14	49±12
MN (pg/ml)	34±13	34±12	36±13	37±11	35±11	35±13	35±13
b: Insulin tolerance test							
	Pre-test	15 min	30 min	45 min	60 min	90 min	120 min
Glycemia (mmol/l)	4.9±0.7	2.4±1.0	1.8±0.8	2.8±0.9	5.0±1.6	6.5±2.7	6.8±2.6
NE (pg/ml)	293±122	326±151	530±197	680±167	583±204	434±153	372±122
NMN (pg/ml)	42±12	44±14	47±21	60±28	65±22	54±27	46±18
E (pg/ml)	43±37	84±76	722±384	600±345	273±351	59±42	57±43
MN (pg/ml)	23±10	33±18	62±45	98±50	101±62	66±41	34±26

NE: Norepinephrine, NMN: Normetanephrine, E: Epinephrine, MN: Metanephrine

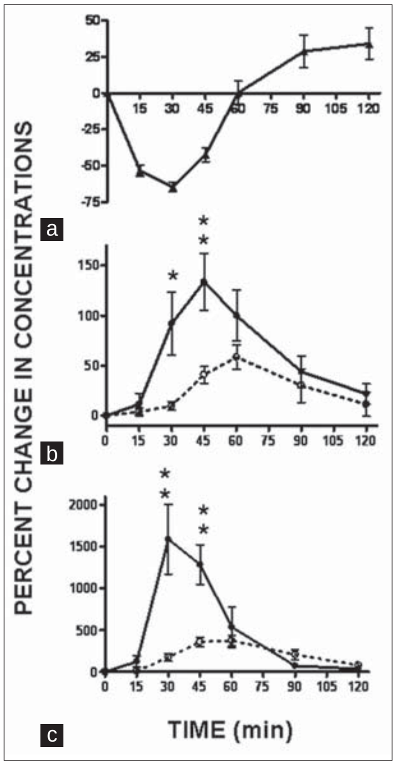

Figure 3: Changes from pre-test values in plasma glucose, catecholamines and metanephrines (MNs) (mean ± SEM) after insulin-induced hypoglycemia test ([a] glycemia, [b] norepinephrine [continuous line] and normetanephrine [broken line], [c] epinephrine [continuous line] and MN [broken line]). ***P*< 0.01 and **P*< 0.05, catecholamine versus MN

101 ± 62 pg/ml ($P < 0.001$) at 60 min and 66 ± 41 pg/ml ($P < 0.01$) at 90 min after insulin injection. The time to peak was later for MN than for epinephrine (54 ± 13 min vs. 41 ± 10 min, respectively $P = 0.003$). The time-course of epinephrine and MN are expressed in percent changes of pretest values and are illustrated in Figure 3c.

DISCUSSION

Stresses stimulate at various degrees the sympathetic nerves traffic and the adrenomedullary hormonal system. As expected, cold pressure test and orthostatic stimulation caused a significant increase mainly in NE levels, while a massive release of epinephrine was the main response to hypoglycemia [10,11].

Upright posture is a well-established and potent stimulus for release of NE by sympathetic nerves leading to a rapid pooling of peripheral blood. The decrease in central venous pressure unloads cardiopulmonary baroreceptors which elicit an increase in sympathetic tone and subsequently in NE [14-16]. In our patients, postural change stimulated sympathetic outflow with a rise of 61% and 28% in plasma NE and epinephrine, respectively. For other sympatho-excitatory tests such as cold pressor test, isometric handgrip, or mental stress, the catecholamine responses were in general similar and quite small [10]. The cold stimulus increases sympathetic outflow by activation of thermal and nociceptor afferents from the immersed hand. In agreement with previous studies, NE and epinephrine levels increased after the cold exposure by 34 ± 12% and 16 ± 7%, respectively [13,16-18]. Insulin-induced hypoglycemia is a potent stimulus eliciting the release of epinephrine from the adrenal medulla and to a lesser extent that of NE from sympathetic neurons and adrenal medulla [11]. In agreement with previous studies [14,19], epinephrine overwhelmingly increased by 1587% about 15 min after the maximal hypoglycemia. Although showing qualitatively similar changes, NE increased only by 134% at the same time.

Whatever the nature of the sympathetic stimulation, the peak concentration of 3-O-methylated derivatives occurs 10-15 min later than that of the catecholamines. In response to postural change, plasma NMN and MN only increased by 30% and 22%, respectively [Figure 1]. These latter modifications are in agreement with those reported after change from supine to sitting (30% and 27%, respectively) [20]. Moreover, 5 min after change from the supine to an upright position, Eisenhofer reported that plasma NMN increases at a lesser extent than plasma NE (27% vs. 130%) [21]. After cold exposure, the rise in NMN levels was attenuated (19 ± 9%, $P < 0.01$) without any detectable change in MN levels [Figure 2]. In response to hypoglycemia, the rise of methylated derivatives was attenuated (326% and 54% for MN and NMN, respectively) compared to that of catecholamines [Figure 3]. Our study confirms previous one showing that plasma MNs are relatively insensitive to large increases in adrenal release of catecholamines [9].

The increase in plasma MNs is attenuated compared to that of catecholamines confirming that only a fraction of released catecholamines are converted by tissular catechol-o-methyl transferase (COMT) [9]. Conversely, in patients with pheochromocytoma, catecholamines are metabolized within the tumor that overexpresses COMT. MNs exceed catecholamines in plasma explaining the better sensitivity of MNs over catecholamines determination for pheochromocytoma screening [4].

Nevertheless, sympathetic and adrenomedullary stimulations diversely increased plasma MNs and may altered the specificity of the screening test. Orthostatic stimulation increased both NMN and MN plasma levels that exceeded the threshold of the biochemical test for 16% and 4% of the patients, respectively. Although the small number of volunteers limits the generalization of the results, cold exposure seems to have few impacts on the performance of the test and MNs levels remained below the threshold. After hypoglycemic stress, plasma levels of MN and NMN exceeded the threshold of the test for 76% and 12% of patients, respectively. This exceeding of the threshold is time-limited and lasts about half an hour.

It is well-established that the high diagnostic sensibility can only be guaranteed with blood sampling under supine and fasting conditions. Applying the upper limit value determined from our patients in standing position, the sensitivity of the diagnostic test is altered. Blood samples should be collected from patients at rest for at least 30 min and in supine rather than in seated position [3,22].

In conclusion, the moderate sympatho-excitatory stimuli such as exposure to cold have little influence on the plasma concentrations of MNs. In contrast, orthostatic and metabolic stressors can lead to falsely positives interpretations. In order to limit false positive values, blood samples for MNs determination should be collected following an overnight fast and after at least 1½ in supine rest.

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