

Anorexia nervosa depends on adrenal sympathetic hyperactivity: opposite neuroautonomic profile of hyperinsulinism syndrome

Fuad Lechin^{1,2}
Bertha van der Dijs^{1,2}
Betty Pardey-Maldonado¹
Jairo E Rivera¹
Scarlet Baez¹
Marcel E Lechin³

¹Department of Pathophysiology, Sections of Neuroendocrinology, Neuropharmacology, and Neurochemistry, Instituto de Medicina Experimental, Faculty of Medicine, Universidad Central de Venezuela, Caracas; ²Instituto de Vías Digestivas Caracas, Centro Clínico Profesional, Caracas, Venezuela; ³Department of Internal Medicine, Texas A and M Health Science Center, College of Medicine, Texas, USA

Objective: The aim of our study was to determine the central and peripheral autonomic nervous system profiles underlying anorexia nervosa (AN) syndrome, given that affected patients present with the opposite clinical profile to that seen in the hyperinsulinism syndrome.

Design: We measured blood pressure and heart rate, as well as circulating neurotransmitters (noradrenaline, adrenaline, dopamine, plasma serotonin, and platelet serotonin), using high-performance liquid chromatography with electrochemical detection, during supine resting, one minute of orthostasis, and after five minutes of exercise. In total, 22 AN patients (12 binge-eating/purging type and 10 restricting type) and age-, gender-, and race-matched controls ($70 \pm 10.1\%$ versus $98 \pm 3.0\%$ of ideal body weight) were recruited.

Results: We found that patients with AN had adrenal sympathetic overactivity and neural sympathetic underactivity, demonstrated by a predominance of circulating adrenaline over noradrenaline levels, not only during the supine resting state (52 ± 2 versus 29 ± 1 pg/mL) but also during orthostasis (67 ± 3 versus 32 ± 2 pg/mL, $P < 0.05$) and after exercise challenge (84 ± 4 versus 30 ± 3 pg/mL, $P < 0.01$).

Conclusion: Considering that this peripheral autonomic nervous system disorder depends on the absolute predominance of adrenomedullary C1 adrenergic nuclei over A5 noradrenergic pontine nucleus, let us ratify the abovementioned findings. The AN syndrome depends on the predominance of overwhelming adrenal sympathetic activity over neural sympathetic activity. This combined central and autonomic nervous system profile contrasts with that registered in patients affected by hyperinsulinism, hypoglycemia, and bulimia syndrome which depends on the absolute predominance of neural sympathetic activity.

Keywords: anorexia nervosa, adrenal sympathetic activity, adrenaline, noradrenaline, eating disorders

Introduction

Anorexia nervosa (AN) is a disorder of unknown etiology characterized by restricting eating and a relentless pursuit of thinness. There is a narrow range of age of onset (early adolescence), stereotypic presentation of symptoms and course, and relative gender specificity. Patients with AN and bulimia have a number of endocrine abnormalities which can be interpreted as adaptations to starvation.^{1,2}

Individuals with AN have an ego-syntonic resistance to eating and a powerful pursuit of weight loss, yet are paradoxically preoccupied with food and eating rituals to the point of obsession. Individuals have a distorted body image and, even when emaciated,

Correspondence: Fuad Lechin
Apartado 80 983, Caracas 1080-A,
Venezuela
Tel +58 212 961 1048
Fax +58 212 961 0172
Email flechin@telcel.net.ve

tend to see themselves as “fat”, express denial of being, and compulsively exercise to excess. They are often resistant to treatment, and lack insight regarding the seriousness of the medical consequences of their disorder. In addition to the above, the relative contributions of psychologic and/or physiologic factors to the appearance and development of the clinical symptoms included into the diagnostic criteria according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) are not well defined. These criteria include two types of AN, ie, the restricting type, in which the person is not regularly engaged in binge-eating or pursuing behavior, and the binge-eating/purging type. Furthermore, individuals with AN display motor restlessness. We investigated the autonomic nervous system and circulating neurotransmitters of 22 patients (12 binge-eating/purging type and 10 restricting type) referred to our institute for assessment, in an effort to find possible neuropharmacologic therapeutic strategies for this disease.

Methods

The study was conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained after the purpose, nature, and potential risks had been explained to the subjects. The experimental protocol was approved by the ethical committee of the Fundación Instituto Medicina Experimental.

Patients

The study included 22 female AN patients (10 restricted type and 12 binge-eating type) and a group of age-, gender-, and race-matched controls. The diagnoses were made according to DSM-IV criteria. Mean age \pm standard deviation (SD) of the AN patients was 22 ± 6.4 years and weight $70 \pm 10.1\%$ of ideal body weight, according to Metropolitan Life Insurance Company tables. All patients and controls were extensively evaluated (physically, endoscopically, radiologically, biochemically, bacteriologically, and immunologically) in order to rule out any other physical illness. Exclusion criteria included pregnancy, lactation, smoking, and alcohol abuse. Neither patients nor controls took any medication for 15 days prior to the beginning of the study.

Measurement of blood pressure (BP) and heart rate (HR) as well as drawing of blood samples were performed simultaneously. Supine BP measurements were taken in a standardized fashion using appropriate-sized cuffs and a random-zero mercury sphygmomanometer. All measurements were taken in accordance with a previously published protocol.³

Blood samples for plasma neurotransmitter determinations were obtained simultaneously with BP and HR measurements through a heparinized catheter, inserted into the antebraial contralateral vein 15 minutes before the first BP and HR measurements. Plasma noradrenaline, adrenaline, dopamine, free serotonin (f5-HT) and platelet serotonin (p5-HT) levels were measured during supine rest, one minute of orthostasis, and after five minutes of moderate exercise.³ All tests were performed on subjects after 10 hours of fasting. A physician in constant attendance noted any symptoms reported by the subjects.

Analytic methods

Noradrenaline, adrenaline, dopamine, plasma f5-HT, and p5-HT levels were measured. For all parameters, the samples were assayed in duplicate and all determinations were made simultaneously. We used reverse-phase, ion-pair high performance liquid chromatography with electrochemical detection for the measurement of monoamines. Optimization of chromatographic conditions and attainment of adequate quantification parameters allowed us to maximize sensitivity and reproducibility.

Blood for catecholamine and serotonin assays was transferred to plastic tubes, each containing 20 mg of ethylenediaminetetraacetic acid (EDTA) and 10 mg of sodium bisulphite/mL in solution. The tubes were carefully inverted and placed on ice. The blood was promptly centrifuged at 600 rpm for 15 minutes at 4°C in order to obtain platelet-rich plasma. Two milliliters of platelet-rich plasma, obtained for determination of p5-HT, were taken and stored at -70°C until assayed. The remaining blood was again centrifuged at 7000 rpm. The supernatant, platelet-poor plasma, was divided into two portions for determination of catecholamines and f5-HT, after which the portions were stored at -70°C until assayed.

Reagents and standards

Noradrenaline, adrenaline, dopamine, serotonin creatinine sulfate, dihydroxybenzylamine, sodium octyl sulfate, dibutylamine, acid-washed aluminum oxide, KH_2PO_4 , citric acid, and EDTA were purchased from Sigma-Aldrich (St Louis, MO). Microfilters were purchased from Whatman Inc. (Florham Park, NY) through Merck SA, (Caracas, Venezuela). Acetonitrile and 2-propanol were obtained from Merck SA. Glass-distilled water was deionized and filtered through a Millipore Milli-Q reagent grade water system (Bedford, MA). Solvents were filtered through a 0.2 μm Millipore filter and were vacuum de-aerated. Standard

solutions (1 mmol/L) were prepared in 0.1 mol/L perchloric acid and diluted to the desired concentration.

Equipment

Liquid chromatography was performed using a Waters 515 HPLC pump (Waters Milford, MA) equipped with a Rheodyne valve injector 7125i, which was fitted with a 50 μ L sample loop (Rheodyne; Berodine, Berkeley, CA). A 15 cm \times 4.6 mm inner diameter Discovery C18 column packed with octadecyl silane 5 μ m particles was preceded by a column prefilter of 2 μ m porosity, both from Supelco/Sigma-Aldrich. The detection system was a 460 electrochemical detector (Waters Corporation, Milford, MA). The potential of the glass carbon working electrode was set at ± 0.61 V versus the silver–silver chloride (Ag–AgCl) reference electrode for detection of catecholamines and 0.70 V versus the Ag–AgCl for detection of indolamines. The chromatograms were registered and quantified using Empower software from Waters Corporation. The results were corrected for the volume of EDTA added.

Analytical assays

Plasma catecholamines

The assay was performed by extraction of the catecholamines onto 20 mg of alumina followed by elution with 200 μ L of 1.0 mol/L HClO₄ using regenerated cellulose microfilters of 0.2 μ m pore size purchased from Whatman Inc. We calibrated the instrument with standard plasma; after incubation with acid-washed aluminum oxide, a plasma pool of free catecholamines was processed similarly to the plasma samples, but 20 μ L of a standard solution of noradrenaline, adrenaline, and dopamine (50, 25, and 25 ng/mL, respectively) was added to the plasma pool. Both the standard plasma and the sample plasma were supplemented with 20 μ L of internal standard (100 ng/mL of dihydroxybenzylamine). The mobile phase was KH₂PO₄ 6.8045 g/L, EDTA 0.1 g/L, and di-N-butylamine 100 μ L/L. Sodium octyl sulphate was added as an ion-pair agent at a concentration of 0.6125 g/L, with the pH adjusted to 5.6. The flow rate was 0.4 mL/min. The sensitivities of this method for noradrenaline, adrenaline, and dopamine, respectively, were 6.4, 5.8, and 2.0 pg/mL. The intra-assay coefficients of variation were 2.8, 4.0, and 4.0%, respectively. The interassay coefficients of variation were 6.7, 4.5, and 4.3%, respectively.

Plasma indolamines

After sonication of platelet-rich plasma to disrupt the platelets (Ultrasonic Liquid Processor, Model 385; Heat Systems Ultrasonics Inc., Farmingdale, NY), both platelet-rich and

platelet-poor plasma were processed in the same way, ie, 200 μ L of 3.4 mol/L perchloric acid and 50 μ L of 5-hydroxytryptophan solution (114.5 μ g/mL) as internal standard, were added to 1 mL of plasma vortexed and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was filtered through a 0.22 μ m membrane (Millipore) and 10 μ L was injected into the column. Calibration runs were generated by spiking blank platelet-poor plasma with 50 μ L of a solution containing 5-HT (10 μ g/mL) and 50 μ L of 5-hydroxytryptophan (114.5 μ g/mL). This standard plasma was processed in the same manner as the samples. The mobile phase was citric acid 3.8424 g/L, sodium acetate 4.1015 g/L, EDTA 0.100 g/L, di-N-butylamine 100 μ L/L, and 30 mL/L of 2-propanol. Sodium octyl sulphate was added as an ion-pair agent in a concentration of 4.25 mg/L with a pH of 5.0. The flow rate was 0.610 mL/min. The sensitivity of the method for serotonin was 0.1 ng/mL. The intra-assay coefficients of variation for p5-HT and f5-HT were 6.2 and 8.7%, respectively.

Statistical methods

Results are presented as the mean \pm standard error of measurement (SEM). Multivariate one-way analysis of variance (ANOVA) with repeated measurements, and correlation coefficients (exploratory factor analysis) were used. Dbase Stats (TM) by Ashton Tate and Statview SE \pm Graphics by Abacus were used for the statistical analysis.

Results

We did not find any significant differences between the two clinical types of AN in our patients.

Cardiovascular parameters

Neither systolic BP nor diastolic BP showed significant variations during orthostasis or after moderate exercise in any group. However, differential pressure showed a significant increase during orthostasis in the AN group. HR showed significant and progressive rises during both orthostasis and exercise periods in the AN group but not in controls (Table 1).

Catecholamines

Plasma noradrenaline showed significant and progressive increases during orthostasis and exercise in the two groups. However, the noradrenaline values and their increases were significantly higher in controls than in the AN patients. In addition, adrenaline showed important and significant increases during orthostasis and exercise in the AN patients, but not in controls. Plasma dopamine levels showed a

Table 1 Systolic blood pressure, diastolic blood pressure, heart rate, noradrenaline, adrenaline, dopamine, platelet-serotonin, and free serotonin blood values, at 0' (resting), 1' (orthostasis) and 5' (post-exercise) in 22 patients with anorexia nervosa and their controls

		0 minutes	1 minutes	5 minutes	P values		
					0' vs 1'	0' vs 5'	1' vs 5'
SBP	- AN	152 ± 5	158 ± 3	171 ± 6	<0.05*	<0.02*	<0.02*
	- C	123 ± 5	124 ± 6	123 ± 2	n.s.	n.s.	n.s.
DBP	- AN	60 ± 3	60 ± 2	61 ± 3	n.s.	n.s.	n.s.
	- C	80 ± 4	77 ± 1	75 ± 3	n.s.	n.s.	n.s.
HR	- AN	71 ± 3	79 ± 4	83 ± 6	<0.02**	<0.01**	<0.001**
	- C	66 ± 2	67 ± 2	70 ± 4	n.s.	n.s.	n.s.
NA	- AN	166 ± 4	169 ± 5	175 ± 5	n.s.	n.s.	n.s.
	- C	178 ± 2	202 ± 4	223 ± 5	<0.05*	<0.01**	<0.01**
AD	- AN	52 ± 2	67 ± 3	84 ± 4	<0.05*	<0.001***	<0.001***
	- C	29 ± 1	32 ± 2	30 ± 2	n.s.	n.s.	n.s.
DA	- AN	18 ± 1	21 ± 2	23 ± 2	n.s.	n.s.	<0.05*
	- C	17 ± 2	18 ± 2	19 ± 3	n.s.	n.s.	n.s.
p5-HT	- AN	228 ± 19	249 ± 22	225 ± 25	n.s.	n.s.	n.s.
	- C	282 ± 21	292 ± 25	276 ± 30	n.s.	n.s.	n.s.
f5-HT	- AN	3.2 ± 1	13.7 ± 1	24.5 ± 2	<0.001***	<0.001***	<0.001***
	- C	2.99 ± 1	1.8 ± 1	3.3 ± 1	n.s.	n.s.	n.s.

Notes: Results are expressed as mean ± SEM. *P < 0.05; **P < 0.02, ***P < 0.001. Most decimals were omitted.

Abbreviations: AD, adrenaline (in pg/mL); AN, anorexia nervosa; C, controls; DA, dopamine (in pg/mL); SBP, systolic blood pressure (in mmHg); DBP, diastolic blood pressure (in mmHg); HR, heart rate (in beats/minute) p5-HT, platelet serotonin (in ng/mL); f5-HT, free serotonin (in ng/mL); NA, noradrenaline (pg/mL); n.s., non significant; SEM, standard error of measurement.

significant increase during orthostasis in AN patients but not in controls.

Indolamines

p5-HT did not show any significant variation in any group. Plasma f5-HT, (ie, outside the platelets) showed mean basal values which were greater in AN patients than in controls and showed progressive and significant increases during orthostasis and exercise in AN patients but not in controls. Significant correlations amongst the different physiologic and neurochemical variables during rest, orthostasis, and after moderate exercise are shown in Table 2.

Discussion

The results presented in this study demonstrate that AN patients have adrenal sympathetic overactivity, as shown by the low noradrenaline:adrenaline plasma ratio registered during orthostasis and exercise testing.⁴ The fact that falls in the noradrenaline:adrenaline ratio were opposed by systolic BP and HR rises fits well with the hypothesis that the basal adrenal sympathetic overactivity which underlies this syndrome was accentuated throughout the stress challenge. This adrenal sympathetic hyperactivity is responsible for the f5-HT rises also registered in these patients, and should be attributed to the increase in adrenaline plasma levels which provokes platelet aggregation,⁵ as revealed by the close positive correlation

between adrenaline and f5-HT levels seen in this study. In addition, we did not find significant physiologic or neuroautonomic differences between the two types of AN patients.

Table 2 Significant correlations (r) for physiologic and plasma neurotransmitter parameters at 0 minutes (resting), one minute (orthostasis), and five minutes (post-exercise) in 22 anorexia nervosa patients and their controls

	SBP	DBP	HR	NA	AD	DA	p5-HT	f5-HT
AN 0 min								
HR	0.61*							
AD	0.66*		0.70*					
DA					0.64*			
f5-HT			0.70*		0.70*			
C 0 min								
HR	0.61*							
AN 1 min								
HR	0.60*							
AD	0.60*		0.69**					
DA					0.63**			
f5-HT			0.60*		0.69*			
C 1 min								
AD			0.60*					
AN 5 min								
HR	0.70**							
AD	0.75**		0.80***					
f5-HT			0.66*		0.68*			

Notes: *P < 0.05; **P < 0.02; ***P < 0.001.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NA, noradrenaline; AD, adrenaline; DA, dopamine; p5-HT, platelet serotonin; f5-HT, free serotonin.

These findings contrast with the enhancement of the noradrenaline:adrenaline ratio and diastolic BP rises registered in both controls and patients affected by hyperinsulinism syndrome.^{6,7} We have demonstrated that this syndrome is associated with maximal enhancement of neural sympathetic activity and hypoglycaemia.⁶⁻⁹ These phenomena depend on both central and peripheral autonomic nervous system mechanisms. The former depends on A5 noradrenergic axons which inhibit C1 adrenergic medullary neurons, whereas the latter should be attributed to the direct inhibitory effect of sympathetic nerves at the adrenal gland level.¹⁰⁻¹³

The above findings are reinforced by the demonstration that a small oral dose of clonidine (an alpha-2 agonist) is able to reverse peripheral (plasma) noradrenaline:adrenaline ratio enhancement.¹⁴ It should be borne in mind that although the adrenergic medullary nuclei may also be inhibited by clonidine, this effect is only registered in mammals with adrenal sympathetic overactivity (minimized noradrenaline:adrenaline plasma ratio). This effect can be attributed to the fact that although the drug is an alpha-2 agonist (preferentially), it also acts at imidazole receptors which crowd adrenal medullary neurons but not A5 neurons.¹⁵⁻²² These facts are reinforced by the observation that other imidazole agonists (rilmenidine, lofexidin) which do not act at the A5 neurons, display a powerful agonistic inhibitory effect at the medullary nuclei.¹⁸⁻²⁶

Some additional information helps to understand the central and peripheral autonomic nervous system interactions which underlie the two opposite syndromes, ie, AN and hyperinsulinism. Oral glucose is absorbed at the small bowel and reaches the pancreatic beta cells which secrete insulin and send inhibitory drive (via gamma aminobutyric acid, GABA) to alpha cells. Plasma insulin crosses the blood-brain barrier and excites the A5 neurons,^{8,9} which send inhibitory axons to the adrenal medullary nuclei.¹⁰ Plasma glucagon also crosses the blood-brain barrier and excites the adrenal nuclei.^{27,28} At the peripheral level, sympathetic nerves which innervate alpha cells, but not beta cells, excite additional release of glucagon into the plasma. Finally, the A5 and the adrenal nuclei interchange inhibitory axons and, thus, predominance of A5 is responsible for neural sympathetic activity, hyperinsulinism, hypoglycemia, and bulimia, whereas overactivity of the other neuroendocrine circuitry results in adrenal sympathetic hyperactivity, hyperglucagonism, hyperglycemia, and anorexia.^{29,30}

With respect to all the above, it should be noted that although the thoracic sympathetic nerves that innervate pancreatic islet cells depend on the adrenal nuclei, lumbar

sympathetic nerves which are responsible for muscular activity depend on the A5 pontine nucleus. This latter branch of peripheral neural sympathetic activity is responsible for circulating noradrenaline plasma levels.³¹⁻³⁴ It should also be noted that the adrenal medullary nuclei are responsible for adrenal gland secretion which depends on thoracic sympathetic preganglionic axons.²⁶ These nuclei receive excitatory axons from other pontomedullary nuclei (acetylcholinergic, serotonergic, and glutamatergic), that are responsible for adrenal gland secretion, which is positively correlated with glucogenolysis and hyperglycemia.³⁵ Special mention should be made about the excitatory drives which arise from the dorsal raphe 5-HT axons (highly associated with stress mechanisms and hyperglycemia), the abrogation of which with tianeptine (a serotonin uptake enhancer drug), is associated with reduction not only of serotonin but also of plasma adrenaline, glucagon, and glucose levels and, in addition, triggers increased insulin secretion.³⁶⁻⁴⁰ Thus, the hyperactivity of the C1 adrenal and dorsal raphe 5-HT axis should underlie the AN syndrome, which also includes raised plasma levels of both adrenaline and f5-HT.^{25,26,41}

The gastric paralyzation and hypotony seen in AN patients contrasts with the fast gastric emptying and hypertony present in subjects affected by hyperinsulinism and hypoglycemia.^{6,7} Radiologic investigation of these subjects always shows a hypertonic steer-horn stomach and an open pylorus which results in fast emptying.⁴² In addition, these patients also show increased plasma noradrenaline and decreased plasma adrenaline levels (very high noradrenaline:adrenaline plasma ratio), which is consistent with the predominance of neural over adrenal sympathetic peripheral branch.⁶

The understanding of the above-mentioned pathophysiologic mechanisms enables adequate neuropharmacologic therapy for both disorders. Whereas patients affected by hyperinsulinism and hypoglycemia can be successfully treated with drugs that minimize neural sympathetic activity,⁷ abrogation of the adrenal sympathetic branch by drugs which minimize the C1 adrenal medullary and dorsal raphe (5-HT) axis, eg, buspirone,⁴¹ amantadine,^{43,44} and/or tianeptine,^{36,37,45} would be able to reverse clinical and radiologic symptoms in AN patients.²⁶

At the peripheral level, AN patients showed raised levels of f5-HT and decreased levels of p5-HT. This is consistent with the increased platelet aggregation known to be triggered by overflow of adrenaline.⁴⁶ Plasma f5-HT released from platelets inhibits insulin release from beta cells.⁴⁷⁻⁵⁰ In addition, plasma f5-HT, but not p5-HT excites the area postrema medullary nucleus (located outside the blood-brain barrier),

which is crowded with 5-HT₃ excitatory receptors (Bezold Harish reflex).^{51,52} Excitation of this mechanism provokes vomiting that is suppressed by ondansetron (a 5-HT₃ antagonist). The area postrema is the only 5-HT medullary nucleus which sends excitatory axons to the adrenal nuclei.^{53,54} Thus, these central and peripheral serotonergic mechanisms would be also annulled by amantadine. Finally, the C1 adrenal nuclei also receive glutamatergic excitatory drive from the hypothalamic area,⁵⁵ that may be intercepted at this level by the drug, which explains its therapeutic effects based on the minimization of adrenal sympathetic hyperactivity.^{7,26,43} Our findings fit well with the decreased affinity of platelet alpha₂ receptors and an adrenaline inhibitory effect as demonstrated by Heufelder et al.⁵⁶

Disclosure

The authors report no conflicts of interest in this work.

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