

Anorexia nervosa versus hyperinsulinism: therapeutic effects of neuropharmacological manipulation

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Background: We have demonstrated that anorexia nervosa is underpinned by overwhelming adrenal sympathetic activity which abolishes the neural sympathetic branch of the peripheral autonomic nervous system. This physiological disorder is responsible for gastrointestinal hypomotility, hyperglycemia, raised systolic blood pressure, raised heart rate, and other neuroendocrine disorders. Therefore, we prescribed neuropharmacological therapy to reverse this central and autonomic nervous system disorder, in order to normalize the clinical and neuroendocrine profile.

Methods: The study included 22 female patients with anorexia nervosa (10 restricted type, 12 binge-eating type) who received three months of treatment with amantadine 100 mg/day. We measured blood pressure, heart rate, and circulating neurotransmitters, (noradrenaline, adrenaline, dopamine, platelet serotonin, free plasma serotonin) during supine resting, one minute of orthostasis, and a five-minute exercise test before and after one, two, and three months of treatment with amantadine, a drug which abrogates adrenal sympathetic activity by acting at the C1(Ad) medullary nuclei responsible for this branch of the peripheral sympathetic activity.

Results: We found the amantadine abolished symptoms of anorexia nervosa from the first oral dose onwards. Normalization of autonomic and cardiovascular parameters was demonstrated within the early days of therapy. Abrupt and sustained increases in the plasma noradrenaline:adrenaline ratio and disappearance of abnormal plasma glucose elevation were registered throughout the three-month duration of the trial. Significant and sustained increases in body weight were documented in all cases. No relapses were observed.

Conclusion: We have confirmed our previously published findings showing that the anorexia nervosa syndrome depends on the hypomotility of the gastrointestinal tract plus hyperglycemia, both of which are triggered by adrenal sympathetic hyperactivity. The above neuroendocrine plus neuroautonomic and clinical disorders which underpinned anorexia nervosa were abruptly suppressed since the first oral dose of amantadine, a drug able to revert the C1(Ad) over A5(NA) pontomedullary predominance responsible for adrenal and neural sympathetic activity, respectively.

Keywords: amantadine, anorexia nervosa, adrenal sympathetic activity, hyperglycemia, hyperinsulinism, neural sympathetic activity

Introduction

We have demonstrated that doxepin, a drug which inhibits the uptake of serotonin (5-HT) is able to normalize patients affected by the hyperinsulinism plus hypoglycemia syndrome.¹ We have also shown that amantadine, a N-methyl-D-aspartate (glutamate) antagonist, is able to enhance insulin secretion and lower plasma glucagon levels.²⁻⁸ Considering that both the metabolic and hormonal effects are paralleled by the

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attenuation of neural sympathetic and adrenal sympathetic activity, respectively, we inferred that neuropharmacological drugs able to attenuate the hyperactivity of the C1(Ad) medullary nuclei³⁻⁸ might be powerful tools for treating adrenal sympathetic predominance, including the anorexia nervosa syndrome.

In view of these observations, along with our previous demonstration that the anorexia nervosa syndrome (both restricted type and binge-eating type) is underpinned by maximal adrenal sympathetic overactivity,⁹ we tested the potential therapeutic effects of amantadine, a N-methyl-D-aspartate antagonist that interferes with excitatory glutamate axons at the medullary C1(Ad) nuclei in patients affected by this pathophysiological disorder.

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 patients with anorexia nervosa (10 restricted type and 12 binge-eating type) before treatment⁹ and after three months of treatment with amantadine 100 mg/day. The diagnoses were made according to Diagnostic and Statistical Manual of Mental Disorders Fourth Edition criteria. The mean age \pm standard deviation of the patients was 22 ± 6.4 years and body weight was $70 \pm 10.1\%$ of ideal weight, according to Metropolitan Life Insurance Company tables. All patients had been extensively evaluated (physically, endoscopically, radiologically, biochemically, bacteriologically, and immunologically) to rule out any other physical illness, as explained in our previously published study.⁹ Exclusion criteria included pregnancy, lactation, smoking, and alcohol abuse. The patients did not take any medication for 15 days prior to the beginning of the study.

Measurement of blood pressure and heart rate, as well as drawing of blood samples, were performed simultaneously. Supine blood pressure 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.¹⁰ Blood samples for plasma neurotransmitter determination were obtained simultaneously with blood pressure and heart rate

measurements through a heparinized catheter inserted into the contralateral antebrachial vein 15 minutes before the first blood pressure and heart rate measurements. Plasma noradrenaline, adrenaline, dopamine, free serotonin (f5-HT), and platelet serotonin (p5-HT) levels were assessed during supine resting, one minute of orthostasis, and after five minutes of moderate exercise. All tests were performed on the subjects after 10 hours of fasting. A physician was in constant attendance, and 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 ethylenediamine tetra-acetic acid and 10 mg of sodium bisulfite/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 ethylenediamine tetra-acetic acid 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 deaerated. 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 Corporation, Milford, MA) equipped with a Rheodyne valve injector 7125i, which was fitted with a 50 μ L sample loop (Rheodyne, 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). The potential of the glass carbon working electrode was set at ± 0.61 V versus the 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 (Waters Corporation). The results were corrected for the volume of ethylenediamine tetra-acetic acid 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 (Piscataway, NJ). 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 (dihydroxybenzylamine 100 ng/mL). The mobile phase was KH₂PO₄ 6.8045 g/L, ethylenediamine tetra-acetic acid 0.1 g/L, and di-N-butylamine 100 μ L/L. Sodium octyl sulfate 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, were 6.4, 5.8, and 2.0 pg/mL, respectively. 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 samples 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 the

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-HT 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, ethylenediamine tetra-acetic acid 0.100 g/L, di-N-butylamine 100 μ L/L, and 30 mL/L of 2-propanol. Sodium octyl sulfate was added as an ion-pair agent at 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

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

Results

We have previously demonstrated that there are no significant neuroendocrine or neuroautonomic differences between the two clinical types of anorexia nervosa, ie, the restricted and binge-eating types.⁹

Cardiovascular parameters

Neither systolic nor diastolic blood pressure showed significant variations during orthostasis or after moderate exercise in our patients. However, differential pressure showed a significant increase during orthostasis before but not after amantadine treatment. Heart rate showed significant and progressive rises during both orthostasis and exercise periods before but not after amantadine treatment (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 after amantadine therapy. In addition, adrenaline showed important and significant increases during orthostasis and exercise in the patients before treatment but not in treated patients. Plasma dopamine levels showed a

Table 1 Systolic, diastolic blood pressure, heart rate, noradrenaline, adrenaline, dopamine, platelet serotonin, and free blood serotonin blood values, at 0 minutes (resting), one minute (orthostasis), and five minutes (postexercise) in 22 patients with anorexia nervosa during a symptomatic period and during an asymptomatic period three months after treatment with amantadine 100 mg/day

	0 min	1 min	5 min	P values		
				0 vs 1 min	0 vs 5 min	1 vs 5 min
SBP ANs	152 ± 5	15 ± 3	17 ± 6	<0.05*	<0.02*	<0.02*
ANa	120 ± 3	12 ± 4	13 ± 6	ns	ns	ns
DBP ANs	60 ± 3	60 ± 2	61 ± 3	ns	ns	ns
ANa	74 ± 4	70 ± 4	76 ± 6	ns	ns	ns
HR ANs	71 ± 3	79 ± 4	83 ± 6	<0.02**	<0.01**	<0.01**
ANa	66 ± 6	64 ± 4	74 ± 6	ns	ns	ns
NA ANs	166 ± 4	169 ± 5	175 ± 5	ns	ns	ns
ANa	186 ± 6	214 ± 8	235 ± 7	<0.05*	<0.01**	<0.01**
Ad ANs	52 ± 2	67 ± 3	84 ± 4	<0.05*	<0.001***	<0.001***
ANa	27 ± 5	30 ± 4	35 ± 3	ns	ns	ns
DA ANs	18 ± 1	21 ± 2	23 ± 2	ns	ns	<0.05*
ANa	15 ± 4	20 ± 4	22 ± 5	ns	ns	ns
p5-HT ANs	228 ± 19	249 ± 22	225 ± 25	ns	ns	ns
ANa	258 ± 31	316 ± 27	308 ± 32	ns	ns	ns
f5-HT ANs	3.2 ± 1	13.7 ± 1	24.5 ± 2	<0.001***	<0.001***	<0.001***
ANa	2.1 ± 1	2.3 ± 1	3.1 ± 1	ns	ns	ns

Notes: Values are mean ± standard error of the mean. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.001$.

Abbreviations: ANa, asymptomatic of anorexia nervosa; ANs, symptomatic of anorexia nervosa; DBP, diastolic blood pressure (mmHg); SBP, systolic blood pressure (mmHg); HR, heart rate (beats/min); NA, noradrenaline (pg/mL); Ad, adrenaline (pg/mL); DA, dopamine (pg/mL); p5-HT, platelet serotonin (ng/mL); f5-HT, free serotonin (ng/mL); ns, not significant.

significant increase during orthostasis in nontreated patients but not in treated patients.

Indolamines

p5-HT did not show any significant variation before or after treatment. Plasma f5-HT, (ie, outside the platelets) showed mean basal values which were greater before treatment and showed progressive and significant decreases after amantadine treatment. Significant correlations between the different physiologic and neurochemical variables during rest, orthostasis, and after moderate exercise are shown in Table 2.

Discussion

Our results show that a low dose (100 mg/day) of amantadine, a drug which abruptly suppresses adrenal sympathetic activity,^{9,11} was able to abolish symptoms of anorexia nervosa in 22 affected patients when it was administered 45 minutes before the main meal, and also when it was administered 45 minutes before an oral glucose tolerance test (manuscript in preparation). Patients were able to eat all types of foods and recovered their normal body weight. Normalization of cardiovascular parameters (enhancement of diastolic blood pressure and reduction of systolic blood pressure and heart rate) were registered in all cases. Namely, the present research

confirms that the anorexia nervosa syndrome is underpinned by an overwhelming predominance of adrenal sympathetic branch activity which abrogates neural sympathetic activity. This pathophysiological disorder includes symptoms at all levels (cardiovascular, digestive, metabolic, endocrine, respiratory, etc), as well as psychological and neuroautonomic disturbances.

Understanding of the pathophysiology of anorexia nervosa syndrome requires knowledge about hyperactivity of the adrenal sympathetic branch^{9,12,13} which is responsible for gastrointestinal hypoactivity,^{9,14-17} systolic blood pressure and heart rate enhancement, hyperglycemia, tracheobronchial dilation + hypersecretion, and anxiety.¹⁸ Neuroendocrine and metabolic disorders should also be included into this syndrome, the source of which is located at the medullary C1(Ad) nuclei responsible for the peripheral adrenal sympathetic branch.^{19,20} The findings presented in this study demonstrate also that an oral dose of amantadine, a N-methyl-D-aspartate antagonist which annuls the firing activity of the C1(Ad) medullary nuclei,¹¹ minimizes systolic blood pressure and heart rate, both of which are cardiovascular parameters positively correlated with adrenal sympathetic activity. The recovery of gastrointestinal motility and normal feeding were paralleled by a body weight increase. These clinical and physiological parameters

Table 2 Significant correlations for physiological and plasma neurotransmitter parameters at 0 minutes (resting), one minute (orthostasis), and five minutes (postexercise) in 22 patients with anorexia nervosa during a symptomatic pretreatment period and during an asymptomatic period three months after treatment with amantadine 100 mg/day

	SBP	DBP	HR	NA	Ad	DA	p5-HT	f5-HT
ANs 0 min								
HR	0.61*							
Ad	0.66*		0.70*					
DA					0.64*			
f5-HT			0.70*		0.70*			
ANa 0 min								
HR	0.63*							
ANs 1 min								
HR	0.60*							
Ad	0.60*		0.69**					
DA					0.63**			
f5-HT			0.60*		0.69*			
ANa 1 min								
Ad			0.63*					
ANs 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: ANa, asymptomatic of anorexia nervosa; ANs, symptomatic of anorexia nervosa; DBP, diastolic blood pressure (mmHg); SBP, systolic blood pressure (mmHg); HR, heart rate (beats/min); NA, noradrenaline (pg/mL); Ad, adrenaline (pg/mL); DA, dopamine (pg/mL); p5-HT, platelet serotonin (ng/mL); f5-HT, free serotonin (ng/mL).

were paralleled by normalization of the insulin versus glucagon balance throughout the oral glucose tolerance test.²

The dramatic therapeutic effect triggered by amantadine supports our hypothesis that the drug acts through central nervous system mechanisms. With respect to this, we now summarize some information dealing with this issue. The C1(Ad) medullary and the A5(NA) pontine nuclei are the motor centers of the adrenal and neural sympathetic branches of the peripheral autonomic system, respectively.³⁻⁸ Glutamate axons excite the former but not the latter nuclei. However, both nuclei interchange inhibitory axons which act at postsynaptic alpha-2 inhibitory receptors.³⁻⁸ The C1(Ad) nuclei send polysynaptic drives to both pancreatic A cells (secreting glucagon) and to the adrenal glands (secreting adrenaline).²¹ Conversely, A5(NA) neurons send polysynaptic drives to the lumbar sympathetic neurons which excite sympathetic ganglia (whose axons release noradrenaline preferentially and dopamine). In addition, insulin released from B cells crosses the blood-brain barrier and excites the A5(NA) neurons,^{3-5,22-25} whereas glucagon secreted from A cells crosses the blood-brain barrier and excites the C1(Ad) nuclei.²⁶⁻³⁰

Circulating insulin triggers hypoglycemia and enhances gastrointestinal motility and feeding, whereas glucagon provokes hyperglycemia and abrogates gastrointestinal motility, both of which are factors responsible for anorexia.^{1,9,19,23,31}

This central and autonomic neuroendocrine interaction facilitates the understanding of the dramatic annulment of anorexia nervosa syndrome provoked by a small oral dose of amantadine, a drug that interrupts the central nervous system C1(Ad) and A cell (glucagon) crosstalk.

Some additional information should facilitate the understanding of the above issue. We were able to demonstrate that insulin crosses the blood-brain barrier and excites the noradrenergic neurons responsible for peripheral neural sympathetic activity,²² and our findings have been confirmed by other authors.²⁵ Furthermore, we have also demonstrated that postprandial hypoglycemia and hyperinsulinism are provoked by the predominance of this sympathetic branch. Furthermore, we found that neuropharmacological manipulation to attenuate the neural sympathetic predominance was able to normalize this postprandial hypoglycemia and hyperinsulinism disorder.^{1,23} These findings are now complemented by the results presented here, showing that the anorexia nervosa syndrome is located on the opposite “side of the coin”, namely, adrenergic hyperactivity and hyperglycemia, which may be abrogated by adequate neuropharmacological manipulation.

Disclosure

The authors report no conflicts of interest in this work.

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