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Central serotoninergic response to orthostatic challenge in patients with neurocardiogenic syncope

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Aims To determine whether central serotoninergic system activity is impaired by orthostatic challenge in patients with neurocardiogenic syncope (NCS).
Methods and results Thirty-five [mean age: 24 (SD): 6 years] patients with a clinical history of NCS and positive head-up tilt test and 35 age-matched healthy volunteers (CON = 25 ± 5 years) with negative response were studied. Overnight dexamethasone suppression test (DST) (1.5 mg given at 11 p.m.) was performed to assess the sensitivity of the hypothalamic-pituitary-adrenal axis by measuring next day cortisol (μg/dL) at 8 a.m. and 4 p.m. Cardiac autonomic function, cortisol, and prolactin (ng/dL) were also determined at baseline supine (BAS) and after 5, 10, and 15 min of orthostatic stress (OS) at 60°. No significant differences were observed in cortisol plasma levels after the DST: CON = 0.6 ± 0.6 μg/dL vs. NCS = 0.6 ± 0.5; $P = 0.7$. Cardiac autonomic function, cortisol, and prolactin responses were similar in both study groups (CON vs. NCS; $P > 0.05$) during BAS: cortisol = 8.6 ± 4 vs.8.7 ± 4 μg/dL and prolactin = 16.8 ± 9 vs. 16.8 ± 9 ng/dL; OS-5: cortisol = 8.7 ± 5 vs. 8.5 ± 4 μg/dL and prolactin = 16.9 ± 9 vs. 15.8 ± 9 ng/dL; OS-10: cortisol = 8.5 ± 5 vs. 8.1 ± 3 μg/dL; prolactin = 16.2 ± 9 vs. 15.8 ± 9 ng/dL, and OS-15: cortisol = 9.0 ± 5 vs. 8.4 ± 4 μg/dL; prolactin = 17.1 ± 9 vs. 15.5 ± 9 ng/dL.
Conclusion Central serotoninergic response during orthostatic challenge was not impaired in patients with recurrent NCS. These findings suggest that the activation of the hypothalamic-pituitary-adrenal axis is not altered in patients with recurrent NCS.

Introduction

Neurocardiogenic or vasovagal syncope (NCS) is the most common cause of recurrent fainting in the general population.[1] Despite its prevalence, the pathophysiology and treatment approach remain widely controversial.[2–5] Several investigators have recently suggested that central serotonergic system pathways participate in the regulation of blood pressure and heart rate. Brain stem regions including the nucleus tractus solitarius and the anterior hypothalamic preoptic region are involved in cardiovascular autonomic control and contain a dense population of serotonergic neurons.[6] Serotonin infusions into the brain inhibit sympathetic neural outflow during acute haemorrhage in rats and result in hypotension and bradycardia similar to that triggered in NCS.[7]

Acute increases in plasma levels of cortisol and prolactin have been documented in patients with vasovagal response induced by head-up tilt test, suggesting an increased responsiveness of the central serotonergic system to orthostatic challenge.[8]

Clinical evidence supporting the role of the central serotonergic system in patients with NCS has been provided by the reduction in the recurrence of syncope in a small-randomized placebo controlled trial with Paroxetine.[9] However, the role of serotonin in modulating reflex responses during orthostatic challenge has not been systematically assessed in patients with NCS. The aim of this study is to determine whether central serotoninergic system responsiveness to a subthreshold orthostatic challenge is impaired in patients with recurrent NCS previously provoked by tilt test.

Methods

Study population

Thirty-five consecutive patients with previous history of NCS (>2 syncope episodes within the last 12 months) and a previous

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positive head-up tilt test were recruited from the Syncope Clinic at the Fundacion Cardiovascular de Colombia. A thorough clinical evaluation, 12-lead ECG and echocardiogram were performed when clinically indicated. Neurological assessment was ordered when indicated (EEG, brain scan). Thirty-five healthy age and gender-matched volunteers with no history of syncope and a negative head-up tilt test were used as a control group (CON). All patients provided verbal and written informed consent that was approved by the institutional Research Ethics Board.

Diagnostic head-up tilt test protocol

Subjects were tilted from the supine position to a 60° upright position using an electrical driven tilt table with footboard support 20 min after venous cannulation. Continuous ECG and beat-to-beat blood pressure monitoring were recorded (Finapress 2300, Ohmeda, USA, now TNO, Amsterdam, The Netherlands). Subjects were tilted-up to 60° for 15 min; if syncope or pre-syncope was not induced, either sublingual nitroglycerin, 400 mg, or low-dose isoprenaline was administered as previously reported.[10] A positive test was defined as syncope or pre-syncope associated with a decrease in systolic arterial blood pressure (SAP, mmHg) <70 mmHg with or without associated bradycardia. Haemodynamic variables were digitized and stored in an IBM-PC compatible microcomputer using CAFTS (Medikro, Oulu, Finland). Responses were classified according to the VASIS classification as: (1) mixed, (2) cardioinhibitory, and (3) vasodepressor.[11]

Study protocol

All subjects were studied at the Autonomic Physiology Laboratory of the Fundacion Cardiovascular de Colombia between 8 a.m. and 12 noon under controlled temperature (20°C) after an overnight fast. Participants abstained from consuming beverages containing xanthines or smoking the day before evaluation. An intravenous line was inserted into a peripheral vein, and subjects were placed in the supine position for 20 min. Continuous ECG monitoring (lead II) and non-invasive recording of blood pressure by continuous beat-to-beat recordings were performed with a Colin Pilot 9200 device (Colin Medical, San Antonio, TX, USA). Data were digitized and stored in an IBM-PC compatible computer using a signal acquisition system DATAQ 720-WINDAQ PRO+ (DataQ Instruments, Akron, OH, USA) and winCPRS (Absolutely Aliens, Finland) for all data analysis. After 20 min in the supine position, all participants were subjected to a subthreshold orthostatic challenge test. Subjects were tilted up to 60° for 15 min, no drug challenges were used because the purpose of this test was not to confirm the susceptibility to NCS.

Neuroendocrine response

After a 10 min baseline recording of heart rate (bpm) and SAP, diastolic arterial blood pressure (DAP, mmHg), and mean arterial blood pressure (MAP, mmHg), a 5 cc blood sample (baseline supine, BAS) was drawn from the right arm to determine plasma levels of cortisol (μg/dL) and prolactin (ng/dL). All subjects were subsequently tilted up to 60° as previously described. Haemodynamic variables were measured and averaged from minute 5 to 10 during orthostatic stress (OS). Blood samples were also drawn at 5, 10, and 15 min during OS to determine cortisol and prolactin plasma levels as an index of dynamic central serotoninergic activation. Plasma was obtained after 15 min of centrifugation and fractioned in aliquots that were stored at −70°C for posterior analysis. Hormone levels were measured with a commercially available radioimmunoassay kit (Elecsis 1010, Roche, Mannheim, Germany).

Autonomic function testing

Heart rate and blood pressure variability

Heart rate and blood pressure variability were also analysed during both BAS and OS. Stabilization period was recorded from 5 to 10 min during OS. The following heart rate variability indexes were calculated: the square root of the mean squared differences of successive RR interval (RMSSD, ms), as well as the RR interval total power spectrum (RRI-TPS, ms^2), normalized low frequency and high frequency bands (RRI-nuLF, Hertz (Hz) and RRI-nuHF, Hz) and sympathovagal balance (RRI-LF/HF).[12] Systolic blood pressure variability was also analysed using fast Fourier transform: SAP total power spectrum (SAP-TPS, $mmHg^2$), normalized low frequency band (SAP-nuLF, Hz) and normalized high frequency band (SAP-nuHF, Hz).[13]

Cardiovagal reflex response

The deep breathing test was performed during controlled respiration at 6 brpm. Heart rate was recorded and the difference between maximal and minimal heart rate for each of the six cycles was calculated and averaged to obtain the inspiratory/expiratory difference (DBD) in bpm (normal value ≥ 15 bpm).[14]

Arterial baroreflex sensitivity (BRS) was assessed after random administration of vasoactive drugs using the Oxford modified technique.[15] A 150 μg IV bolus of phenylephrine followed after 60 s by a 100 μg IV bolus of sodium nitroprusside was administered. The baroreflex slope was calculated using a linear correlation between the changes in RR interval (increasing or decreasing) after the preceding change in systolic arterial pressure. Only correlations >0.8 were accepted for analysis.[15]

Dexamethasone suppression test

In a different session, the low-dose overnight dexamethasone suppression test (DST) was performed to suppress the adrenocorticotropic hormone secretion and evaluate the hypothalamic-pituitary-adrenal axis.[16] All subjects took 1.5 mg of oral dexamethasone at 11 p.m. on the evening before neuroendocrine assessment. Plasma samples were taken at 8 a.m. and 4 p.m. the following day for the analysis of cortisol 'post-dexamethasone'. Lack of suppression of cortisol was determined with plasma levels <5 μg/dL at either 8 a.m. post-dexamethasone or 4 p.m. post-dexamethasone. The detection limit for cortisol levels was below 0.7 μg/dL and the intra- and inter-assay coefficients of variation were below 5 and 7%, respectively.

Statistical analysis

Data are presented as means and standard deviation (SD). Continuous variables were subjected to a Shapiro–Wilk test to determine whether the data fitted normal distribution. Group comparisons were analysed using either Student's *t*-test (normal distribution) or Wilcoxon rank-sum (abnormal distributions). A $P < 0.05$ value was considered statistically significant.

Results

No significant differences were observed in demographic, baseline haemodynamic variables and biochemical markers between groups (*Table 1*). Subjects with NCS had an average of 5.1 ± 3.8 syncope attacks in the year prior to the inclusion in the study. Positive responses to the diagnostic head-up tilt test in patients with NCS were classified according to VASIS classification[11] as follows: 23 mixed, 5 cardioinhibitory, and 7 vasodepressor responses. Mean time to the vasovagal response was 19.3 ± 7.4 min. All the control subjects had a negative tilt test.

Cardiac autonomic function

Vasovagal response was induced in 12 (35%) patients with NCS during the 15 min of OS with a mean time to syncope of 8.4 ± 3.0 min. Nine patients (75%) had a mixed response, two (16%) a vasodepressor response, and one (9%) a cardio-inhibitory response. Conversely syncope was not induced in the control group and in 23 of the NCS patients. Orthostatic stress vasovagal responders were significantly younger than negative responders (20.9 ± 5.1 vs. 25.8 ± 6.0 years, $P = 0.01$).

No significant difference was observed in heart rate, blood pressure, heart rate variability, blood pressure variability, deep breathing test, or BRS during BAS and during OS recording comparing the two groups (*Table 2*). Furthermore, no significant differences were observed in cardiac autonomic function testing comparing NCS responders, NCS non-responders and controls, respectively.

Table 1 Demographic characteristics

Variables	CON ($n = 35$)	NCS ($n = 35$)
Age (mean, SD years)	25.3 (51)	24.1 (6.1)
Gender (male, %)	11 (31)	11 (31)
BMI (mean, SD kg/m^2)	23.2 (4.0)	22.5 (3.1)
HR (mean, SD b.p.m.)	69 (10.3)	65 (10.1)
SAP (mean, SD mmHg)	109 (14.4)	107 (11.8)
DAP (mean, SD mmHg)	57 (10.3)	55 (9.8)
MAP (mean, SD mmHg)	76 (10.8)	73 (9.3)
Haemoglobin (mean, SD mg/dL)	14.1 (1.35)	14.1 (1.2)
Haematocrit (mean, SD %)	42.3 (3.5)	42.5 (3.7)
FPG (mean, SD mg/dL)	84.8 (9.03)	89.4 (71)
Serum creatinine (mean, SD mg/dL)	0.9 (0.1)	0.9 (0.1)
Cholesterol (mean, SD mg/dL)	182.9 (34.9)	189.9 (42.1)
Triglycerides (mean, SD mg/dL)	103.5 (65.7)	114.6 (89.6)
HDL (mean, SD mg/dL)	41.5 (13.2)	45.0 (7.7)

HR, heart rate (beats per minute); BMI, body mass index; HDL, high density lipoprotein; FPG, fasting plasma glucose; CON, controls; NCS, neurocardiogenic syncope.

Neuroendocrine response

Cortisol and prolactin responses were similar in the two groups (CON vs. NCS; $P > 0.05$); BAS: cortisol = 8.6 ± 4 vs. 8.7 ± 4 μg/dL and prolactin = 16.8 ± 9 vs. 16.8 ± 9 ng/dL, 5 min OS: cortisol = 8.7 ± 5 vs. 8.5 ± 4 μg/dL and prolactin = 16.9 ± 9 vs. 15.8 ± 9 ng/dL; 10 min OS: cortisol = 8.5 ± 5 vs. 8.1 ± 3 μg/dL and prolactin = 16.2 ± 9 vs. 15.8 ± 9 ng/dL as well as during 15 min OS: cortisol = 9.0 ± 5 vs. 8.4 ± 4 μg/dL and prolactin = 17.1 ± 9 vs. 15.5 ± 9 ng/dL. No significant differences were observed in prolactin levels comparing NCS patients with a positive response vs. non-responders and controls during baseline and OS (*Table 3*). However, non-responding NCS subjects had lower cortisol levels at baseline and during OS compared with NCS responders. There were no differences in cortisol levels between controls and responders at baseline and during OS (*Figure 9*).

Dexamethasone suppression test

No significant differences ($P > 0.05$) were observed in cortisol plasma levels during the DST between groups at 8 a.m.: CON = 0.8 ± 0.6 μg/dL vs. NCS = 0.6 ± 0.5 μg/dL, $P = 0.7$ and at 4 p.m.: CON = 0.85 ± 1.49 μg/dL vs. NCS = 0.25 ± 0.33 μg/dL. Both groups had normal responses to the DST (*Figure 2*). No significant differences between NCS responders vs. controls (0.86 ± 0.6L vs. 0.79 ± 0.79 μg/dL were observed.

Discussion

The main finding of this study was that central serotoninergic system response to OS and hypothalamic-pituitary-adrenal axis integrity was not altered in patients with recurrent NCS.

Table 2 Haemodynamic and autonomic variables during laboratory assessment

Variables	CON ($n = 35$)		NCS ($n = 35$)	
	Supine	OS	Supine	OS
HR (mean, SD b.p.m.)	64 (7)	82 (14)	61 (7)	78 (10)
SAP (mean, SD mmHg)	106 (13)	128 (20)	107 (11)	119 ± 21
DAP (mean, SD mmHg)	57 (10)	81 (20)	56 (10)	75 ± 17
MAP (mean, SD mmHg)	74 (10)	94 (16)	73 (9)	88 ± 18
RMSSD (mean, SD ms)	62.2 (29)	64.1 (35)	8.3 (38)	67.9 (41)
RRI-TPS (mean, SD ms^2)	3903.3 (3649)	4334.9 (3883)	3634.8 (2944)	4740.1(3267)
RRI-nuLF (mean, SD nu)	1083.8 (845)	1358.3 (1310)	977.7 (901.7)	1325.4 (903)
RRI-nuHF (mean, SD nu)	1054.4 (908)	1017.4 (906)	1457.8 (1533)	1398.8 (1870)
RRI-LF/HF (mean, SD)	1.44 (1)	1.74 (1)	1.10 (1)	1.7 (1)
SAP-TPS (mean, SD ms^2)	17.0 (24)	45.2 (50)	14.2 (12)	30.4 (34)
SAP-nuLF (mean, SD nu)	3.48 (5)	16.5 (20)	2.45 (2)	10.3 (13)
SAP-nuHF (mean, SD nu)	1.71 (4)	4.84(6)	1.12 (1)	3.51 (4)
RRI-LF/HF (mean, SD)	3.20 (3)	4.31 (4)	2.57 (2)	3.82 (3)
DBD (mean, SD b.p.m)	21.5 (8)	N/A	21.8 (7)	N/A
BRS-PHEN (mean, SD mmHg/ms)	38.8 (8.5)	N/A	36.4 (24.0)	N/A
BRS-NITRO (mean, SD mmHg/ms)	24.4 (14.3)	N/A	28.2 (17.4)	N/A

No statistical difference were found in HR and blood pressure as well as in autonomic function variables, during baseline supine-rest and during OS-S comparing the two groups. N/A, not applicable; CON, controls; NCS, neurocardiogenic syncope. See text for other definitions.

Table 3 Hormonal responses during OS

Variables	CON ($n = 35$)	NCS-Responders ($n = 12$)	NCS-Non responders ($n = 23$)
Cortisol baseline (mean, SD μg/dL)	9.4 (6.2)	11.3 (6.5)	7.4 (2.2)*
Cortisol OS-5 min (mean, SD μg/dL)	9.5 (6.8)	11.1 (6.3)	7.2 (2.6)*
Cortisol OS-10 min (mean, SD μg/dL)	9.3 (6.7)	11.9 (6.1)	7.5 (2.8)*
Cortisol OS-15 min (mean, SD μg/dL)	9.9 (6.5)	N/A	8.6 (2.5)
Prolactin baseline (mean, SD ng/dL)	16.9 (9.6)	16.7 (10.9)	16.2 (16.9)
Prolactin OS-5 min (mean, SD ng/dL)	17.0 (9.2)	17.0 (8.2)	15.8 (10.1)
Prolactin OS-10 min (mean, SD ng/dL)	16.8 (9.4)	16.4 (6.3)	15.7 (10.1)
Prolactin OS-15 min (mean, SD ng/dL)	17.3 (9.0)	N/A	15.3 (9.7)

*$P < 0.05$ comparing NCS-responders vs. NCS-Non responders. CON, controls; NCS, neurocardiogenic syncope.

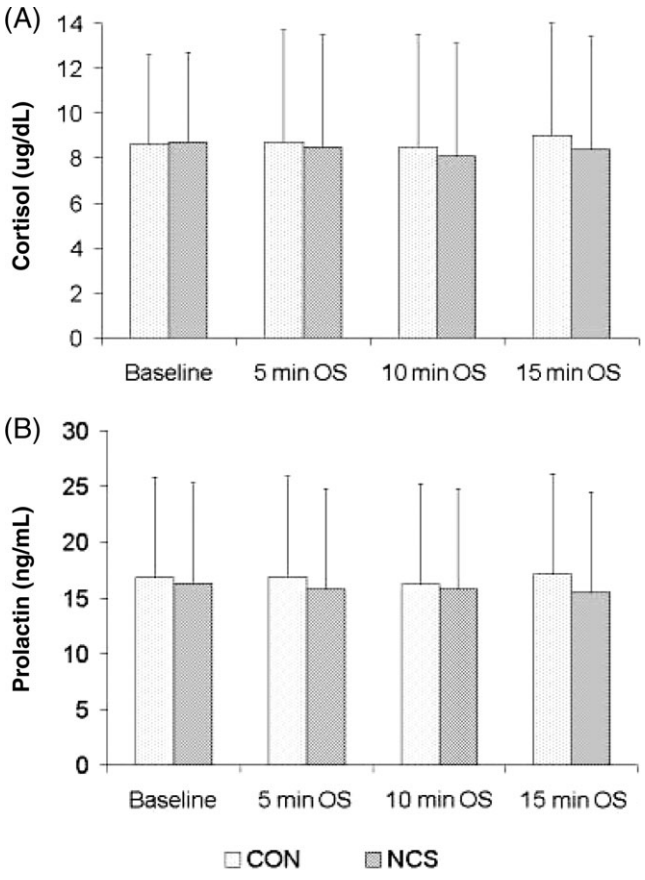

Figure 1 (*A*) Cortisol response during OS; (*B*) prolactin response during OS. No statistical differences were found in baseline levels of cortisol and prolactin between groups as well as during OS. CON, controls; NCS, neurocardiogenic syncope.

Cortisol and prolactin response to orthostatic challenge

We did not observe alterations in the response to orthostatic challenge in cortisol or prolactin regardless of whether syncope was induced (*Figure 1*). These observations suggest that the activation of central serotoninergic system to subthreshold orthostatic challenge is not impaired in patients with recurrent NCS. Other investigators have documented increased prolactin and cortisol responses to clomipramine administration in patients with vasovagal response compared with normal subjects[17] and during head-up tilt test[8] and after syncope response.[18] These investigators concluded that central serotoninergic system mechanisms are involved in the genesis of NCS on the basis of the assumption that a higher hormonal responsiveness to clomipramine and orthostatic challenge were related to central serotoninergic activation. Orthostatic challenge is a powerful stress for susceptible subjects and may induce a myriad of neurohumoral and endocrine alterations that may simply be part of the homeostatic response to the abrupt onset of hypotension and bradycardia. The administration of methylsergide (a serotonin receptor antagonist) significantly reduces the neurohumoral and endocrine alterations triggered by head-up tilt test.[19] However, the reduction of these neuroendocrine alterations was not associated with the prevention of hypotension induced by orthostatic challenge. Although these data suggest that the central serotoninergic system has a role in the genesis of NCS, our findings indicate that prolactin and cortisol secretion are not increased by orthostatic challenge in patients with recurrent NCS.

Dexamethasone suppression test

We performed the DST in all subjects to assess whether the central regulation of adrenocorticotropic hormone was impaired in patients with recurrent NCS. This test can be used as a surrogate of hypothalamic-pituitary-adrenal function. In the presence of an increased central activation of the hypothalamic-pituitary-adrenal axis, the DST should have documented an impaired suppression of cortisol after intervention. This was not the case in our study, indicating that the hypothalamic-pituitary-adrenal axis is not chronically affected in patients with NCS. Support to our findings has been recently provided by Alboni *et al.*[20] These investigators assessed central serotoninergic activity by determining platelet and plasma serotonin levels measured by high-pressure liquid chromatography with electrochemical detection in patients with NCS during tilt test. They did not observe any significant change in serotonin blood levels during tilt. Plasma serotonin did not change significantly at the beginning of the prodrome, during syncope or after recovery of consciousness. The inability to document increased hypothalamic-pituitary-adrenal axis and central serotoninergic activation may be related to the fact that only peripheral measurements were performed in both studies. Similarly, we used DST as a surrogate marker of central hypothalamic-pituitary-adrenal axis activation. However, there is evidence indicating a good

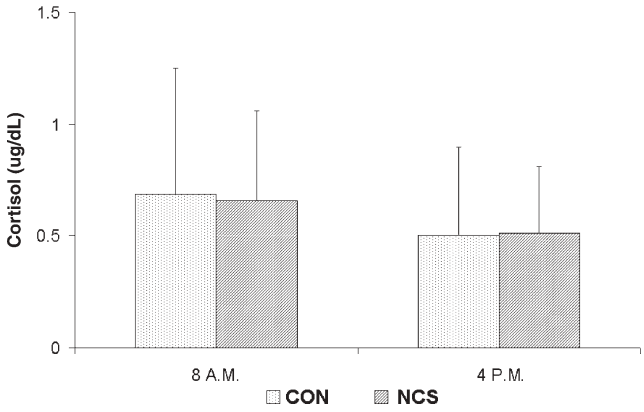

Figure 2 Dexamethasone suppression test. No differences were observed in cortisol levels determinations between groups at 8 a.m. and 4 p.m. Both groups had normal responses to dexamethasone suppression. CON, controls; NCS, neurocardiogenic syncope.

correlation between central and peripheral serotoninergic activity.[21]

Cardiac autonomic response

No significant alterations in baseline cardiac vagal reflexes were documented in NCS patients. Heart rate variability responses were similar in the two groups at baseline and during orthostatic challenge before syncope, which is in agreement with previously reported information.[22–24] Autonomic nervous system alterations may be a sudden event ushered by abrupt withdrawal of sympathetic traffic associated with increase in vagal activity that determines the vasovagal response and may not be detected by the techniques used.[22–24]

Role of serotonin in NCS

A potential role for serotonin (5-HT) regulation and the pathophysiology of NCS has been suggested.[7,8] Previous studies in animal models have documented that intravenous and intracerebral infusions of 5-hydroxytryptophan (5-HT_p) may cause abrupt declines in heart rate, blood pressure, and sympathetic nerve activity.[25,26] Kuhn *et al.*[27] have shown that the central conversion of 5-HT_p to 5-HT seems to be the mechanism by which 5-HT_p exerts its vasodilator effect. However, there are different types of serotonin receptors in the nucleus tractus solitarius. Thus, the stimulation of 5-HT_2 post-synaptic receptors by 5-HT_2 agonist increases blood pressure and sympathetic nerve discharge.[28] Conversely 5-HT_1 receptor stimulation decreases blood pressure and heart rate by a central increase in the vagal tone and the suppression of sympathetic tone. Acute sympathetic withdrawal can be produced by the injection of 5-HT into the intracerebral ventricular areas, suggesting that the vasodilatation and bradycardia seen during vasovagal response may be centrally mediated.[6] Further support for the role of serotonin in patients with NCS has been suggested by some observational studies and a small-randomized trial that reported a significant reduction in syncope recurrence after the administration of serotonin reuptake inhibitors.[5,9,29] It is possible that these medications have alternative mechanisms that are not related with the modulation of the hypothalamic-pituitary-adrenal axis.

Conclusion

Central serotoninergic responses during orthostatic challenge were not impaired in patients with recurrent NCS. These findings suggest that the activation of the hypothalamic-pituitary-adrenal axis is not altered in patients with recurrent NCS during orthostatic challenge.

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