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Assessment of serum level of corticotropin-releasing factor in primary nocturnal enuresis

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Summary

Introduction
Primary nocturnal enuresis is one of the sleep related phenomena characterized by disruption in the relationship between arousal and urination. Corticotropin-releasing factor (CRF) is a neurohormone released from the paraventricular nucleus of the hypothalamus into the median eminence to elicit release of adrenocorticotrophin from the anterior pituitary. It may act to modulate autonomic function and behavior in concert with the endocrine effects. Conflicting animal studies about the role of CRF in micturition, either facilitating or inhibiting, have been raised. It was suggested to be a novel target for treatment of urinary disorders based on the finding that manipulation of CRF in the pontine micturition circuit could affect urodynamic function.

Aim
The aim was to throw light on the possible role of CRF in primary monosymptomatic nocturnal enuresis by assessing its serum level.

Subjects and methods
Twenty-nine children aged 8–14 years complaining of primary monosymptomatic nocturnal enuresis and 16 age- and sex-matched healthy children with good toilet control day and night were recruited to the study. History taking, clinical examination, and assessment of serum CRF levels in the morning and evening (9 a.m. and 9 p.m.) were carried out for all patients and controls.

Results and discussion
A positive family history of enuresis was detected in 82.8% of enuretic patients. Serum levels of CRF (both morning and evening) were significantly lower in patients than in controls. Several animal studies suggested that CRF in descending projections from Barrington's nucleus to the lumbosacral parasympathetic neurons is inhibitory to micturition, which supports our results and the assumption that reduction of the evening serum CRF level could have a role in the occurrence of primary monosymptomatic nocturnal enuresis. No significant difference was found between morning and evening CRF serum levels in either cases or controls, which negates our assumption of having a rhythmic pattern of release (figure). No correlations with age were found. According to their history, all our enuretic patients were deep sleepers. Deep sleep and difficult arousal were found to have a major role in primary monosymptomatic nocturnal enuresis. It was proposed that CRF function may allow arousal to occur before micturition to facilitate preparative behaviors. A lower CRF level may explain deep-sleep pattern in children with enuresis.

Conclusion
CRF was deficient in our enuretic children, which may draw attention to the possible pathophysiological implications in primary nocturnal enuresis (either at the level of loss of inhibitory effect on micturition or lack of arousal in response to bladder distension). Further proof studies are recommended.

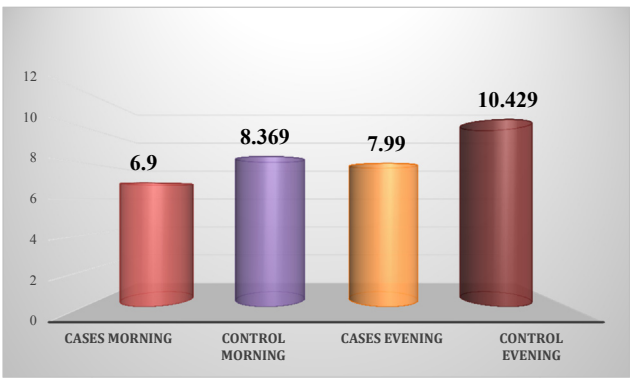

Figure Corticotropin-releasing factor serum levels in cases and controls (morning and evening).

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Introduction

Nocturnal enuresis (NE) refers to discrete episodes of urinary incontinence during sleep in children older than 5 years of age [1]. It can be primary or secondary. It is a stressful condition that needs treatment to relieve anxiety and prevent the accompanying stigma attached to it [2]. Various pathophysiologic mechanisms for primary NE have been discussed as maturational delay, genetic defect, disturbances in vasopressin secretion, bladder capacity defect, or sleep pattern [2–5].

Primary NE is considered to be one of the sleep-related phenomena "parasomnias" [6], characterized by disruption in the relationship between arousal and urination [7]. One factor that plays an important role in coordinating micturition and arousal is corticotropin-releasing factor (CRF) [8]. It is a neurohormone released from the paraventricular nucleus of the hypothalamus into the median eminence to elicit release of adrenocorticotrophin from the anterior pituitary, a hallmark of stress response [9]. CRF gives rise to divergently projecting pathways. It may act to modulate autonomic function and behavior in concert with its endocrine effect [10]. Conflicting animal results regarding the role of CRF in regulation of micturition have been raised: it could be a facilitator by decreasing the micturition threshold and increasing micturition frequency [11]. In contrast, CRF agonists were found to inhibit urination in the awake state and receptor antagonists had the opposite effect [7]. It was suggested to be a novel target for treatment of urinary disorders based on the finding that manipulation of CRF in the pontine micturition circuit could affect urodynamic function [7].

Aim

The aim was to throw light upon the possible role of CRF in primary monosymptomatic NE through assessment of its serum level. No human studies concerning CRF in enuresis were found.

Subjects and methods

Twenty-nine children aged 8–14 years complaining of primary monosymptomatic NE (no urinary control since birth and no other urinary symptoms) attending the outpatient clinic at the National Research Centre were consecutively enrolled in this study (children coming from any part of the country with no specification to any area or social class, and fulfilling the inclusion and exclusion criteria). Exclusion criteria were children less than 6 years of age or more than 18 years, daytime incontinence or urinary symptoms, conditions causing secondary NE (neurological abnormalities, spinal, brain or pelvic surgery, and congenital anomalies of the urologic tract). Other exclusion criteria were urinary tract infection, diabetes mellitus, and diabetes insipidus. All cases had not received any enuresis-related medications for at least 1 year before inclusion in the study. Another 16 healthy children matched for age and sex with good toilet control day and night were recruited as controls.

All cases were subjected to history taking with special emphasis on sleep pattern and voiding history (to differentiate between monosymptomatic and non-monosymptomatic NE), anthropometric measurement (height, weight, and body mass index), clinical examination and investigations (urine, stool analysis, plain X-ray for lumbosacral spines (to exclude spina bifida), and ultrasonography (abdominal and pelvic to assess urinary tract abnormality). Serum CRF was assessed in all patients and controls in the morning and evening (2 mL of venous blood was withdrawn at 9 a.m. and 9 p.m. to test the circadian pattern of secretion (2 controls missed their night sampling). Samples were centrifuged and sera were stored at −20 °C until assayed using an enzyme-linked immunoassay kit (Assay Designs Inc., Ann Arbor, MI, USA).

The study was approved by the ethics committee of the National Research Centre; all patient guardians gave informed written consent.

Statistical analysis

Data were analyzed using the SPSS with statistical package version 15 (SPSS Inc., Chicago, IL, USA). Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. The chi-square test (Fisher exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using the Student *t* test. The Pearson correlation coefficient was used to examine the relationship between two quantitative variables. A p value < 0.05 was considered significant.

Results

Descriptive data and comparison between cases and controls are presented in Table 1. Both groups (patients and controls) were statistically matched.

Comparison between cases and controls regarding CRF serum levels (morning and evening) are presented in Table 2 and Fig. 1. Both morning and evening serum CRF levels were significantly lower in patients than in controls ($p = 0.013$ and $p = <0.01$ respectively).

No significant difference was detected regarding sex in either cases or controls.

The comparison between morning and evening CRF serum levels in each group is presented in Table 3. No statistical significant difference was found between morning and evening levels in either cases ($p = 0.4$) or controls ($p = 0.57$).

Correlation of CRF with age was statistically insignificant in both cases and controls (Table 4).

Discussion

CRF is a neurotransmitter in Barrington's nucleus neurons. These neurons can coregulate the parasympathetic tone of the bladder [12] and brain noradrenergic activity [9] by targeting the preganglionic lumbosacral neurons [12] and projection into the locus ceruleus (LC), which is the major brain norepinephrine nucleus [9]. The LC norepinephrine

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Table 1 Descriptive data and comparison between patients (enuretic children) and controls.

Variables	Patients ($n = 29$)	Controls ($n = 16$)	p
Age (years)	11.64 ± 3.08	9.63 ± 2.94	0.92
Sex (m/f)	18/11 (62.1%/37.9%)	6/10 (37.5%/62.5%)	0.11
Weight (kg)	41.88 ± 17.01	30.09 ± 8.94	0.07
Height (cm)	144.88 ± 1191	127.91 ± 13.46	0.74
Body mass index (kg/m^2)	19.46 ± 5.54	17.94 ± 1.7	0.3
Enuresis during mid-day sleep	10/29 (34.5%)	–	
Deep sleeper by history	29 (100%)	0	
Positive family history	24/29 (82.8%)	–	
Parenteral consanguinity	19/29 (65.5%)	–	
Mode of inheritance AD/AR	9/20 (31%/69%)	–	
Positive urine analysis (crystals: oxalate, urate)	6/29 (20.7%)	3/16 (18.75%),	0.88
Positive stool analysis (*Giardia*/*Entamoeba*)	7/29 (24.1%)	4/16 (25%)	0.95
X-ray lumbosacral	No spina bifida	–	

Note. AD = autosomal dominant; AR = autosomal recessive.
$p < 0.05$ is significant.

system had long been implicated in arousal and attention [13]. LC neurons are activated by diverse stimuli including bladder and colon distension that is translated to forebrain electroencephalographic activation [14,15]. The CRF family may affect various body systems independently, stimulating peripheral CRF receptors via vagal and/or autocrine/paracrine pathways, but data supporting any clinical significance are still limited [16]. CRF was postulated to have an important role in coordinating both micturition and arousal [8]. We assumed that CRF has a role in primary NE.

Table 2 Comparison between cases and controls regarding corticotropin-releasing factor (CRF), morning and evening.

Serum level of CRF (ng/mL)	Group (number)	Mean ± SD	p
CRF morning (ng/mL)	Patients ($n = 29$)	6.9 ± 1.661	0.013
	Controls ($n = 16$)	8.369 ± 4.422	
CRF evening (ng/mL)	Patients ($n = 29$)	7.993 ± 7.091	<0.01
	Controls ($n = 14$)	10.429 ± 13.513	
CRF morning, patients (ng/mL)	Males ($n = 18$)	7.38 ± 1.66	0.19
	Females ($n = 11$)	5.91 ± 1.29	
CRF evening, patients (ng/mL)	Males ($n = 18$)	7.06 ± 0.84	0.09
	Females ($n = 11$)	6.23 ± 1.69	
CRF morning, controls (ng/mL)	Males ($n = 6$)	10.63 ± 6.7	0.1
	Females ($n = 10$)	6.98 ± 1.46	
CRF evening, controls (ng/mL)	Males ($n = 6$)	15.48 ± 20.4	0.2
	Females ($n = 10$)	6.63 ± 1.83	

$p < 0.05$ is significant.

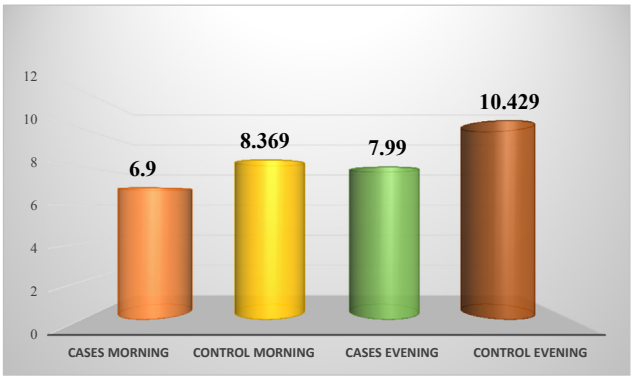

Figure 1 Corticotropin-releasing factor serum levels in cases and controls (morning and evening).

Serum CRF level

A statistically significant reduction was detected in enuretic children compared with controls (both morning and evening). Intrathecal administration of CRF in anaesthetized rats was found to decrease the amplitude of bladder contractions, suggesting that CRF in descending projections from Barrington's nucleus to lumbosacral parasympathetic neurons is inhibitory [17]. Intrathecal or

Table 3 Corticotropin-releasing factor (CRF) (morning vs. evening serum levels) in cases and controls.

Group/number	CRF mean ± SD (ng/mL)	p
Cases morning ($n = 29$)	6.9 ± 1.661	0.4
Cases evening ($n = 29$)	7.993 ± 7.091	
Control morning ($n = 16$)	8.369 ± 4.422	0.57
Control evening ($n = 14$)	10.429 ± 13.513	

$p < 0.05$ is significant.

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Table 4 Correlations of corticotropin-releasing factor (CRF) with age.

Variables		Morning CRF serum level (ng/mL)	Evening CRF serum level (ng/mL)
Age of patients (years)	*r*	−0.12	−0.16
	p	0.54	0.41
Age of controls (years)	*r*	−0.14	−0.22
	p	0.6	0.44
Age of all children studied (years)	*r*	−0.19	−0.25
	p	0.21	0.108

$p < 0.05$ is significant.

systemic administration of CRF receptor 1 (CRF-R1) antagonists in conscious rats, increased urinary frequency and decreased bladder capacity [7]. The inhibitory effect of CRF on micturition reflexes is also supported by La Berge et al. [18]. The previous findings support our results and the assumption that reduction of the evening serum CRF level could have an important role in primary monosymptomatic NE. In contrast, intrathecal or systemic CRF in conscious rats was found to induce bladder overactivity and facilitate micturition [19]. To our knowledge, there are no human studies concerning CRF in NE.

CRF and its receptors play a crucial regulatory role in adaptation to exogenous and endogenous stress stimuli [16]. Acute stress and fear are often associated with urination. However, chronic stress, particularly social stress, results in urinary retention. Chronic stress increases CRF mRNA expression in Barrington's nucleus neurons [20]. This favors the inhibitory influence of CRF on micturition.

CRF receptors are distributed in various tissues [21]. CRF receptor 1 (CRF-R1) is expressed throughout the brain; it is concentrated in the anterior pituitary corticophs and is activated by CRF. It mediates action of CRF at the corticophs and some aspects of behavior stress response, including fear and anxiety. CRF-R2 attenuates behavioral stress responses [22].

Developmental and inflammatory-induced plasticity of CRF-R1 in micturition spinal reflex pathways and supraspinal locations has been demonstrated [18]. An age-dependent upregulation of CRF-R1 in association with preganglionic parasympathetic neurons in the lumbosacral spinal cord has been demonstrated [18]. It has been suggested that upregulation of CRF-R1 in bulbospinal projections to sacral parasympathetic neurons may contribute to mature voiding reflexes and continence [23]. Our results didn't find any correlation with age in either cases or controls (both morning or evening).

Rhythm

A number of neurotransmitters control both positive and negative regulation of CRF release [24]. Norepinephrine, serotonin, corticosteroid [20] and vasopressin have been implicated in its release. It is possible that CRF has a rhythmic pattern of release as it is regulated by vasopressin, which has a circadian rhythm [25] and regulates the rhythmic release of adrenocorticotropic hormone and cortisol. No statistical significant difference was found between morning and evening CRF serum levels in either cases or controls, which negates our assumption of having a rhythmic pattern of release and agreed with Muglia et al. [26], who advocated that "variation of CRF is not required for rhythm generation of cortisol and ACTH."

Arousal

Various animal studies have shown that the state of arousal may or may not have an effect on the micturition reflex and urination [11]. In human studies, a change in arousal state (better or worse) in enuretic children was supported by Imada et al. [27], but Su et al. [28] did not find any difference in arousal between enuretic and non-enuretic children. According to their history, the children with NE in our study were deep sleepers. Deep sleep and difficult arousal were found to have a major role in primary monosymptomatic NE in children [29]. It was proposed that CRF function may allow arousal to occur before micturition to facilitate preparative behaviors [7]. This could explain the deep-sleep pattern of children with enuresis and their lack of arousal in response to a full bladder.

Conclusion

CRF was deficient in our enuretic patients, which can draw attention to the possible pathophysiologic implications in primary NE (either at the level of loss of inhibitory effect on micturition or by lack of arousal in response to bladder distension). This is a preliminary study that cannot prove causation, but we think it is an important finding that needs further research.

Limitations of the study

This study was limited by the small number of cases and controls, but it is just a preliminary study. Also, the male to female ratio between cases and controls was not ideal (but it was statistically insignificant).

Conflict of interest

None.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpurol.2016.08.030.

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