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CLINICAL RESEARCH

Correlations between urinary excretion of catecholamines and electrocardiographic parameters of vagal hyperreactivity in infants with fainting spells. Implication of sympathetic hypotonia?

Corrélations entre les excréctions urinaires des catécholamines et les paramètres électrocardiographiques de l'hyperréactivité vagale chez des nourrissons présentant des malaises. Implication d'une hypotonie sympathique ?

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Abbreviations: ALTE, apparent life threatening events; Cr, creatinine; D, dopamine; E, epinephrine; ECG, electrocardiogram; F min, minimum heart rate; Δ Fi, percentage of deceleration of the heart rate; HR, heart rate; Log, neperian logarithm; NE, norepinephrine; OCR, oculocardiac reflex; RR max, maximum interval between two R waves; SIDS, sudden infants death syndrome; U, urinary excretion; VHR, vagal hyperreactivity.

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KEYWORDS

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 dopamine;
 Oculocardiac reflex;
 Holter ECG;
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 syndrome

MOTS CLÉS

Hyperréactivité
 vagale ;
 Malaises du
 nourrisson ;
 Noradrénaline,
 adrénaline et
 dopamine urinaires ;
 Réflexe
 oculocardiaque ;
 Holter ;
 Syndrome de mort
 subite du nourrisson

Summary

Background. — Vagal hyperreactivity (VHR) is a frequent etiology of infant fainting spells; but it is sometimes difficult to diagnose. A biochemical test would therefore be useful, especially as the oculocardiac reflex (OCR) test innocuity is not absolute.

Aims. — To evaluate urinary excretions of norepinephrine, epinephrine and dopamine as markers for vagal hyperreactivity.

Methods. — During check-up of 55 infants from 0.5 to 11 months of age, for discomfort episodes, including OCR and Holter recording, 24 h urinary assays of total norepinephrine, epinephrine and dopamine were carried out to evaluate sympathetic activity.

Results. — Epinephrine and norepinephrine urinary excretions were negatively correlated with VHR intensity, as measured by the OCR ECG parameters: RRmax, % cardiac deceleration and minimal frequency; dopamine excretion was not. When RRmax_{OCR} was greater or equal to 800 ms, epinephrine urinary excretion tended to be less or equal to 9 nmol/mmol creatinine and norepinephrine excretion less or equal to 190 nmol/mmol creatinine.

Conclusion. — A delay in maturation of the sympathetic system and/or adrenomedullary glands with low secretion of norepinephrine and epinephrine inducing a disequilibrium of the sympathetic/parasympathetic balance may contribute to the fainting spells observed with VHR. Epinephrine and norepinephrine urinary excretions may provide informative complementary noninvasive markers for VHR.

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Résumé

Justification. — L'hyperréactivité vagale (HRV) est une étiologie fréquente des malaises chez le nourrisson; son diagnostic est parfois difficile. Un test biochimique serait donc utile, d'autant que l'innocuité de l'épreuve du réflexe oculocardiaque (ROC) n'est pas absolue.

Objectif. — Répondre à la question: les excrétions urinaires de noradrénaline, adrénaline et dopamine sont-elles marqueurs d'HRV?

Méthodes. — Les excrétions urinaires de noradrénaline, adrénaline et dopamine totales de 24 heures ont été mesurées pour évaluer l'activité sympathique chez 55 nourrissons de 0,5 à 11 mois présentant des malaises et soumis au bilan habituel clinique et électrocardiographique incluant le ROC et l'enregistrement Holter de 24 heures.

Résultats. — Les excrétions urinaires d'adrénaline et noradrénaline sont négativement corrélées avec l'intensité d'HRV appréciée par les paramètres électrocardiographiques du ROC: RRmax, pourcentage de décélération et fréquence minimale cardiaques. Lorsque RRmax au ROC est supérieur ou égal à 800 ms, l'excrétion nyctémérale d'adrénaline tend à être inférieure ou égale à 9 nmol/mmol de créatinine, celle de noradrénaline inférieure ou égale à 190 nmol/mmol de créatinine.

Conclusion. — Un retard de maturation du système sympathique et/ou des médullosurrénales avec hyposécrétion de noradrénaline et d'adrénaline entraînant un déséquilibre de la balance parasympathique/sympathique pourrait contribuer aux malaises associés à une HRV. Les excrétions nyctémérales d'adrénaline et noradrénaline pourraient constituer des marqueurs complémentaires intéressants et non invasifs d'HRV.

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Introduction

Vagal hyperreactivity (VHR) is a frequent etiology in infant fainting spells or in apparent life threatening events (ALTE). It appears isolated, without any other cause of fainting spell, in about 12 to 15% of all cases [1]. It is more frequently found in association with gastro-esophageal reflux. VHR is also associated with reflex anoxic seizures (white breath-holding) [2]. It is suspected to participate in the etiology of sudden infant death syndrome (SIDS) [3–7].

Even though VHR is that frequent and systematically investigated for infant discomfort evaluation in our centre of infant cardiology, it is still difficult to diagnose and even controversial. Diagnosis relies on a stack of clinical,

historical and electrocardiographic arguments. Two complementary tests currently used for that purpose in infants are oculocardiac reflex (OCR) and 24-h Holter ECG [4,6,7]. But normal limits for these two tests, particularly for OCR, are discussed and falsely negative results were reported with secondary evidence of VHR [4]. Furthermore, the innocuity of OCR is not absolute. Besides infant relapsing fainting spells with VHR may be treated by atropinic drugs [8]. We thought that complementary biochemical tests exploring the sympathetic and parasympathetic nervous systems would be welcome for the diagnosis, the follow-up under treatment and a better comprehension of the physiopathology of VHR. Since noninvasive, urinary tests are preferred in infants.

In a previous experimental study, we observed a decrease in norepinephrine (NE) urinary excretion (U) in rats with experimental vagal hypertonia induced by reserpine. Opposite variations were found in a model of vagal hypotonia induced by diphemanil-methylsulfate [9].

We therefore determined UNE, but also U epinephrine (E) and U dopamine (D) in infants with fainting spells who were tested for VHR.

Subjects and methods

Subjects

Fifty-five infants from 0.5 to 11 months of age were investigated at the "Centre de cardiologie infantile du Château-des-Côtes" for VHR, 51 after fainting spells, four as siblings of SIDS victims. The investigation includes routinely, besides the history and the clinical examination, OCR and 24-h Holter ECG [6]. At the end of the evaluation, diagnosis was established, ranging from frank VHR (score 4) to absence of VHR (score 1). Positive diagnosis of VHR (score 4) is admitted in the presence of one major criterium, or three minor criteria. Possible VHR (score 3) or doubtful VHR (score 2) are considered in the presence of two minor criteria or one minor criterion respectively.

Major criteria:

- fainting or syncope during the Holter ECG monitoring with a simultaneous prolonged sinus pause;
- inducibility of fainting or syncope during OCR reproducing the same type of faint described by the family.

Minor criteria:

- history of familial VHR;
- identification of vagally mediated signs induced by factors like pain, vomiting, crying;
- clinical symptoms such as pallor, hypotonia, cyanosis of the lips;
- positive OCR test or indexes of VHR on the Holter recording.

The study complied with the Helsinki declaration. After informed and written consent of the parents, as recommended by Huriet Law, two consecutive noninvasive urinary collections were carried out. A urinary bag was installed for a 3-h collection starting between 9 h and 10 h 30 a.m. just after the clinical examination and the OCR test; then a 21-h collection followed immediately and covered the whole night period. Results for 24 h were obtained by calculation. This protocol was established to test the diagnostic value of the 3-h versus the 24-h collection.

Electrophysiological cardiac parameters

The quantitative parameters of OCR were studied as in [5,6,10]:

- minimum heart rate ($F_{min_{OCR}}$), calculated on three successive beats, expressed as beats per minute (bpm);
- $RR_{max_{OCR}}$ (maximum interval between two R waves, in ms);
- ΔFi_{OCR} , percentage of heart rate (HR) deceleration:
 - $\Delta Fi = [(HR \text{ before the test} - HR \text{ during the test}) / HR \text{ before the test}] \times 100$.

Evident VHR criteria under 3 months are: $F_{min_{OCR}} \leq 50$ bpm, $RR_{max_{OCR}} \geq 1200$ ms or $\Delta Fi_{OCR} \geq 66\%$.

Moderate VHR criteria under 3 months are: $F_{min_{OCR}} \leq 75$ bpm, $RR_{max_{OCR}} \geq 800$ ms or $\Delta Fi_{OCR} \geq 50\%$.

The Holter quantitative parameters were [4,6]:

- $F_{min_{Holter}}$, minimum cardiac frequency observed during the 24 h (in bpm);
- ΔFi_{Holter} , maximal deceleration upon 10 s, observed during the 24 h.

VHR criteria are $F_{min_{Holter}} \leq 80$ bpm under 1 month, ≤ 70 bpm under 2 months, ≤ 60 bpm between 2 months and 1 year, or $\Delta Fi_{Holter} \geq 55\%$ or $\Delta Fi_{Holter} \geq 100$ bpm absolute deceleration.

Urinary collection and biochemical assays

Urine collection was carried out after addition of 0.5 mL of HCl for 3 h and 2 mL for 21 h in the storage bottles, kept at 4°C during the collection and frozen at -30°C thereafter. Urine acidity was checked (pH between 1 and 3).

Total norepinephrine, epinephrine and dopamine (free and conjugated) were measured by high performance liquid chromatography (HPLC) in reverse phase with amperometric electrochemical detection, after hydrolysis (20 min at 80°C, at a pH of 0.8 to 1) and extraction by ionic exchange on Biorad column [9].

Creatinine (Cr) was determined by the Jaffé method on KONE "Optima" analyzer. The first 20 samples were measured with and without previous pH neutralization. The results obtained were similar. Therefore pH neutralization was omitted further on.

All results of biochemical assays of catecholamines were expressed as nmol per mmol Cr.

Statistical methods

In the whole group infants from 0.5 to 11 months or in different groups of age, the urinary excretions of the different catecholamines are represented by the mean \pm SD and/or the (range). \log_e of the values was used to normalize their distribution if necessary.

For studying correlations between urinary excretion of each catecholamine and A_{37} age or electrocardiographic parameters, Pearson's correlation as well as multiple correlation coefficients were determined, using the statistical Analysis System SAS 9.2 software (SAS Institute Inc, Cary, NC, USA) with the CORR procedure; adjustment for age was effected by the REG procedure (0 if ≤ 3 months, 1 if > 3 months, since 3 months of age has been found to be a critical milestone, see discussion).

For studying the variations of excretion of each metabolite in function of age (age sections of 1 month) or collection time (3 h/21 h) or VHR score (1 to 4), analysis of variance was used, followed by comparison of means by the Bonferroni-Student *t* test.

The level of significance was considered as $P \leq 0.05$.

Results

Clinical characteristics of the infants

Age

Out of 55 infants, six were under 1 month (0.5 to 0.9 months), 15 from 1.0 to 1.9 months, 18 from 2.0 to 2.9 months, eight from 3.0 to 3.9 months, three from 4.0 to 4.9 months, five older than 5 months (5.3, 7.6, 9.5, 10, 10.3). Five children were preterms, (30 to 36 weeks). Maturation of the cardiac control being in relation with the gestational age [11], we used for these children an A_{37} age, as if they had been born at 37 weeks. Eighty-five percent of the children had their first fainting spell before 3 months of age.

Diagnosis of VHR

Sixteen children had positive VHR (score 4), 15 possible VHR (score 3), nine doubtful VHR (score 2), 15 absence of VHR (score 1). None of the four siblings of SIDS victims had VHR.

Urinary epinephrine

Variations of excretion in function of age or collection time

For the infants of all ages, the mean $UE_{24h} = 14.8 \pm 10.1$ nmol/mmolCr (with high individual variations, from 1 to 43.8 nmol/mmolCr). In contrast with norepinephrine excretion (see below), neither UE_{24h} nor $\text{Log}UE_{24h}$ were correlated with the A_{37} age ($r = 0.077$ and $r = 0$ respectively).

For what concerns the effect of collection time, UE_{21h} was lower than UE_{3h} only in children aged less than 2 months (8.7 ± 5.6 nmol/mmolCr vs 24.4 ± 24.2 nmol/mmolCr, $P < 0.005$). Beyond 2 months of age, the difference disappeared.

Correlations with OCR ECG parameters

In all children between 0.5 and 11 months, $\text{Log}UE_{24h}$ was negatively correlated with RR_{maxOCR} ($r = -0.34$; $P = 0.012$), positively correlated with F_{minOCR} ($r = +0.31$; $P = 0.024$), marginally correlated with ΔFi_{OCR} ($r = -0.24$; $P = 0.084$) (Table 1).

When adjusted for age, the previous correlations were not markedly modified (for example, $P = 0.014$, instead of $P = 0.012$, for the correlation between $\text{Log}UE_{24h}$ and RR_{maxOCR}).

When the three OCR ECG parameters used for the diagnosis of VHR were taken together in a multiple correlation, $\text{Log}UE_{24h}$ was highly correlated with the three parameters ($r = |0.36|$; $P = 0.009$).

Fig. 1 shows the individual results for RR_{maxOCR} and the correlation curve obtained. The latter shows that when RR_{maxOCR} is greater or equal to 800 ms, $\text{Log}UE_{24h}$ tends to be less or equal to 2.2 and consequently UE_{24h} tends to be less or equal to 9 nmol/mmolCr.

Correlations with Holter ECG parameters

We found no correlation between $\text{Log}UE_{24h}$ and the Holter ECG parameters, whatever the age (Table 1).

Table 1 Correlations between urinary catecholamine excretions and electrocardiogram variables during oculocardiac reflex dynamic testing and 24-hour Holter recording in infants with fainting episodes, aged between 0.5 and 11 months.

ECG variables	Correlation coefficient r (95% confidence interval)			Multiple correlation coefficient r
	RR_{max} , ms	F_{min} , bpm	ΔFi (%)	
<i>During oculo-cardiac reflex test</i>				
Epinephrine (Log nmol/mmolCr)	-0.34 (-0.55; -0.08)**	0.31 (0.04; 0.54)*	-0.24 (-0.47; 0.03) ^a	0.36 ***
Norepinephrine (Log nmol/mmolCr)	-0.25 (-0.49; 0.02) ^a	0.19 (-0.08; 0.43)	-0.11 (-0.37; 0.16)	0.33 **
Dopamine (nmol/mmolCr)	0.02 (-0.25; 0.28)	-0.04 (-0.30; 0.23)	0.10 (-0.17; 0.36)	0.17
<i>During 24-hour Holter recording</i>				
Epinephrine (Log nmol/mmolCr)	—	0.16 (-0.11; 0.41)	-0.13 (-0.39; 0.16)	—
Norepinephrine (Log nmol/mmolCr)	—	0.11 (-0.17; 0.36)	0.12 (-0.17; 0.39)	—
Dopamine (nmol/mmolCr)	—	-0.02 (-0.29; 0.25)	0.22 (-0.07; 0.47)	—

Urinary vanillylmandelic acid and homovanillic acid were also determined, but no significant correlations were found with the ECG variables during oculocardiac reflex testing or 24-hour Holter recording.

bpm: beats per minute; ECG: electrocardiogram; ΔFi : percentage of heart rate deceleration; F_{min} : minimum heart rate; RR_{max} : maximum interval between two R waves. * $P \leq 0.05$; ** $P \leq 0.02$; *** $P \leq 0.01$.

^a Marginal significance ($0.06 \leq P \leq 0.10$).

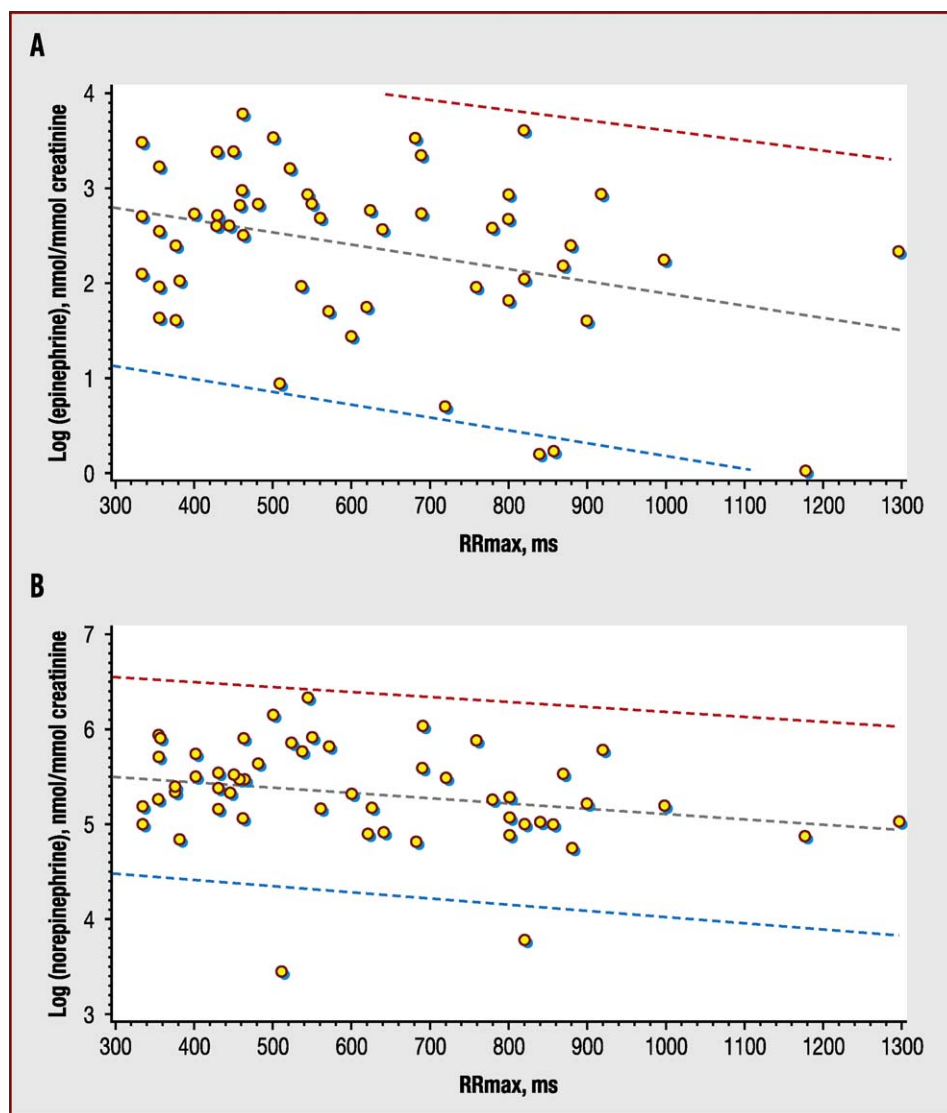


Figure 1. A. Correlation between Log_e (epinephrine) and RRmax on ECG recording during oculocardiac reflex for detecting vagal hyperreactivity, in infants with fainting spells (0.5 to 11 months old). $r = -0.34$; $P = 0.012$. B. Correlation between Log_e (norepinephrine) and RRmax on ECG recording during oculocardiac reflex for detecting vagal hyperreactivity, in infants with fainting spells (0.5 to 11 months old). $r = -0.25$; $P = 0.06$. Log_e (norepinephrine) was significantly correlated with the three OCR ECG parameters of VHR (RRmax, Fmin and % deceleration) taken together in a multiple correlation ($r = |0.33$); $P = 0.015$).

Studies of correlations with UE_{3h}

Studies of correlations with UE_{3h} were unsuccessful, partly because of the great variability of spontaneous urine collection volume, during this short period. The same will apply for UNE_{3h} and UD_{3h} .

Urinary norepinephrine

Variations of excretion in function of age or collection time

For all the infants aged from 0.5 to 11 months, the mean UNE_{24h} was 229 ± 104 nmol/mmolCr (30.0 to 468 nmol/mmolCr); UNE_{24h} was correlated with the A_{37} age ($r = -0.26$, $P = 0.05$); LogUNE_{24h} was more tightly correlated with the A_{37} age ($r = -0.30$, $P = 0.027$).

UNE_{24h} was higher in infants under 3 months (259 ± 107 nmol/mmolCr) than in infants greater or equal to 3.0 months (179 ± 94 nmol/mmolCr), $P < 0.005$.

For what concerns the effect of collection time, in the children under 3 months, UNE_{21h} was lower than UNE_{3h} (248 ± 70 vs 408 ± 197 nmol/mmolCr, $P < 0.001$). Beyond 3 months, the significance of the difference disappeared (180 ± 100 vs 211 ± 105 nmol/mmolCr).

Correlations between UNE_{24h} and OCR ECG parameters

In all the infants from 0.5 to 11 months, LogUNE_{24h} was significantly correlated with the three OCR ECG parameters of VHR taken together in a multiple correlation ($r = |0.33$); $P = 0.015$) (Table 1).

When the OCR ECG parameters were considered separately, $\text{LogUNE}_{24\text{h}}$ was marginally correlated with $\text{RRmax}_{\text{OCR}}$ ($r = -0.25$; $P = 0.06$), but not correlated significantly either with Fmin_{OCR} ($r = +0.19$; $P = 0.17$) or with $\Delta\text{Fi}_{\text{OCR}}$ ($r = -0.11$; $P = 0.41$).

When adjusted for age, the previous correlations were not markedly modified.

Fig. 1 shows the individual results for $\text{RRmax}_{\text{OCR}}$ and the correlation curve obtained. The latter shows that when $\text{RRmax}_{\text{OCR}}$ is greater or equal to 800 ms, $\text{LogUNE}_{24\text{h}}$ tends to be less or equal to 5.24 and $\text{UNE}_{24\text{h}}$ tends to be less or equal to 190 nmol/mmolCr.

Correlations between $\text{UNE}_{24\text{h}}$ and Holter ECG parameters

No correlation was found between $\text{LogUNE}_{24\text{h}}$ and $\text{Fmin}_{\text{HOLTER}}$ or $\Delta\text{Fi}_{\text{HOLTER}}$ (Table 1).

Urinary dopamine

Variations of excretion in function of age or collection time

For the infants of all ages, the mean $\text{UD}_{24\text{h}} = 2651 \pm 1063$ nmol/mmolCr (77 to 6190 nmol/mmolCr). $\text{UD}_{24\text{h}}/\text{Cr}$ did not vary significantly with age. $\text{UD}_{21\text{h}}/\text{Cr}$ was lower than $\text{UD}_{3\text{h}}/\text{Cr}$ until 3 months of age, the difference reaching significance in children between 2 and 3 months ($P < 0.025$).

Correlations with OCR ECG parameters

In all the infants from 0.5 to 11 months, $\text{UD}_{24\text{h}}$ was not correlated with RRmax ($P = 0.90$), with Fmin_{OCR} ($P = 0.76$) or with $\Delta\text{Fi}_{\text{OCR}}$ ($P = 0.47$) (Table 1).

Correlations with Holter ECG parameters

No significant correlation was found between $\text{UD}_{24\text{h}}$ and $\text{Fmin}_{\text{HOLTER}}$ ($P = 0.88$) or $\Delta\text{Fi}_{\text{HOLTER}}$ ($P = 0.13$) in the infants from 0.5 to 11 months (Table 1).

Discussion

The various studies previously published about the urinary excretion of the different catecholamines in children aimed essentially at establishing normal ranges to track down neuroblastomas [12–17]. In this prospect, the authors sought essentially to establish higher limits. No lower limits were generally given. For each catecholamine studied, our results are in agreement with those previously published. We also confirmed a strong interindividual variability. To our knowledge, no study described the evolution of catecholamine urinary excretion in infants under 1 year. Decrease of UNE/Cr after 3 months has not been reported. The circadian differences we observed (higher excretions in the morning during the 3-h collection, before 3 months for NE and D and before 2 months for E) had not been described before. We must notice that the morning time collection corresponded, in our study, to a period of stress: clinical examination, OCR, setting of Holter electrodes and urinary bag. Besides, one must

recall that the infants studied here had presented fainting spell(s).

Our study of the correlations between the quantitative urinary excretion of catecholamines and quantitative ECG parameters in infants with fainting spell(s), is in fact independent of the qualitative diagnosis of VHR in the various individuals, which may be sometimes difficult (in case of scores 2 or 3: possible or doubtful VHR). The ECG parameters measured during the OCR were generally found to be more strongly correlated with urinary excretion of catecholamines than the ECG parameters measured on the Holter recording.

$\text{UE}_{24\text{h}}$ was negatively correlated with VHR intensity, as measured by OCR ECG parameters. Indeed, in the case of frank VHR, Fmin_{OCR} decreases, RRmax and $\Delta\text{Fi}_{\text{OCR}}$ increase. In this situation, we generally observed a diminution of UE. When $\text{UE}_{24\text{h}}$ is less or equal to 9 nmol/mmolCr, RRmax tends to be greater or equal to 800 ms (Fig. 1) and Fmin less or equal to 70 bpm. Low UE could be a useful auxiliary marker for VHR.

$\text{UNE}_{24\text{h}}$ was also negatively correlated with VHR intensity as represented by the three OCR ECG parameters taken together and also particularly by $\text{RRmax}_{\text{OCR}}$ alone. If $\text{UNE}_{24\text{h}}$ is less or equal to 190 nmol/mmolCr, RRmax tends to be greater or equal to 800 ms. Low $\text{UNE}_{24\text{h}}$ could be another marker for VHR.

These results suggest the possible contribution of sympathetic hypotonia and/or adrenomedullary hyposecretion in the physiopathology of faintings associated with VHR.

Our findings are in accordance with our previous experimental observations [9]: diminution of UNE in rats with pharmacological parasympathetic hypertonia (–44%) and increase in UNE in rats with parasympathetic hypoactivity (+61%).

At the critical age of 3 months, $\text{UNE}_{24\text{h}}$ decreases and its significant circadian modifications tend to disappear in our study. At the same period, heart rate starts to diminish, with a more significant reduction during daytime [18]. In this study, the age of 71% of the infants was less or equal to 2.9 months, that of 15% between 3 and 3.9 months; 85% of the infants had their first fainting spell before 3.0 months of age. Moreover, SIDS incidence curve shows a peak at about 3 months. SIDS may be related to VHR. Several cases of SIDS were observed in infants having noradrenergic deficits [19]; these deficits were alterations of catecholaminergic neurons [20–22], alterations in catecholamine enzymes [21,23,24] or alterations in alpha2-adrenergic receptors [25].

For what concerns the possible use of $\text{UE}_{24\text{h}}$ and $\text{UNE}_{24\text{h}}$ as markers for VHR, it must be stressed that it is difficult to determine their sensitivity and specificity since the diagnosis of VHR is greatly clinical. Another difficulty is due to the existence of three classes of infants: one class of infants with positive VHR (28% of the infants with score 4), one class with absent VHR (28% of the infants with score 1), but also a third class with uncertain VHR (44% of the infants with possible or doubtful VHR corresponding to scores 3 or 2); the latter class, which represents almost one half of the whole group of infants, cannot be used for determining a sensitivity and a specificity.

In our study, $\text{UE}_{24\text{h}}$ appeared as a more promising marker than $\text{UNE}_{24\text{h}}$. In the infants from 0.5 to 11 months, the mean $\text{LogUE}_{24\text{h}}$ was 2.74, 2.29 and 2.20 (corresponding to $\text{UE}_{24\text{h}}$ of

15.5, 9.9 and 9.0 nmol/mmolCr) for scores 1, (2 + 3) and 4 respectively (not significant). At the critical age between 1 and 3 months, the mean LogUE_{24h} was 2.73, 2.28 and 2.04 (corresponding to UE_{24h} of 15.3, 9.8 and 7.7 nmol/mmol Cr) for scores 1, (2 + 3) and 4 respectively (not significant); however at this critical age, 50% of infants with score 4 had a UE_{24h} less or equal to 9 nmol/mmolCr whereas no infant with score 1 had a UE_{24h} less or equal to 9 nmol/mmol Cr. A more extensive study is necessary to test the interest of UE_{24h} and UNE_{24h} as markers for VHR.

Conflict of interest statement

No conflict of interest.

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