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

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Received 11 December 2009; received in revised form 30 June 2010; accepted 2 July 2010

Abbreviations: ALTE, apparent life threatening events; Cr, creatinine; D, dopamine; E, epinephrine; ECG, electrocardiogram; F min, minimum heart rate; ΔFi, 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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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 hypotonia induced by reserpine. Opposite variations were found in a model of vagal hypotonia induced by diphenamid-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 criterion, 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 (FminOCR), calculated on three successive beats, expressed as beats per minute (bpm);
- RRmaxOCR (maximum interval between two R waves, in ms);
- ΔFiOCR, percentage of heart rate (HR) deceleration:
  - $\Delta F_i = [(HR \text{ before the test } - HR \text{ during the test})/HR \text{ before the test}] \times 100$.

**Urine 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 excretion of the different catecholamines are represented by the mean ± 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 $A_{27}$ 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 $\leq 3$ 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 Bonferoni-Student t test.

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

$\begin{align*}
\text{Evident VHR criteria under 3 months are:} \\
F_{\text{minOCR}} \leq 50\text{ bpm, } RR_{\text{maxOCR}} \geq 1200\text{ ms or } \Delta F_{\text{iOCR}} \geq 66\%. \\
\text{Moderate VHR criteria under 3 months are:} \\
F_{\text{minOCR}} \leq 75\text{ bpm, } RR_{\text{maxOCR}} \geq 800\text{ ms or } \Delta F_{\text{iOCR}} \geq 50\%. \\
\end{align*}$
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 A37 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 0). Sixteen children had positive VHR (score 4), 15 possible VHR (score 3), nine doubtful VHR (score 2), 15 absence of VHR (score 0). 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 UE24h = 14.8 ± 10.1 nmol/mmolCr (with high individual variations, from 1 to 43.8 nmol/mmolCr). In contrast with norepinephrine excretion (see below), neither UE24h nor variations, from 1 to 43.8 nmol/mmolCr). In contrast with norepinephrine excretion (see below), neither UE24h nor UE21h were correlated with the A37 age (r = 0.077 and r = 0 respectively).

For what concerns the effect of collection time, UE21h was lower than UE24h 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, LogUE24h was negatively correlated with RRmaxOCR (r = −0.34; P = 0.012), positively correlated with FminOCR (r = +0.31; P = 0.024), marginally correlated with ∆FiOCR (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 LogUE24h and RRmaxOCR).

When the three OCR ECG parameters used for the diagnosis of VHR were taken together in a multiple correlation, LogUE24h was highly correlated with the three parameters (r = 0.36; P = 0.009).

Fig. 1 shows the individual results for RRmaxOCR and the correlation curve obtained. The latter shows that when RRmaxOCR is greater or equal to 800 ms, LogUE24h tends to be less or equal to 2.2 and consequently UE24h tends to be less or equal to 9 nmol/mmolCr.

Correlations with Holter ECG parameters
We found no correlation between LogUE24h 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 |
| Epinephrine (Log nmol/mmolCr) | RRmax, ms | Fmin, bpm | ∆Fi (%) | RRmax, Fmin and ∆Fi |
| Epinephrine (Log nmol/mmolCr) | −0.34 (−0.55; −0.08)** | 0.31 (0.04; 0.54)* | −0.24 (−0.47; 0.03)* | 0.36*** |
| Norepinephrine (Log nmol/mmolCr) | −0.25 (−0.49; 0.02)* | 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 |
| Epinephrine (Log nmol/mmolCr) | −0.16 (−0.41) | 0.13 (−0.39; 0.16) | −0.13 (−0.41) | 0.16 |
| Norepinephrine (Log nmol/mmolCr) | −0.11 (−0.36) | 0.12 (−0.39) | 0.12 (−0.39) | 0.39 |
| Dopamine (nmol/mmolCr) | −0.02 (−0.29; 0.25) | 0.22 (−0.07; 0.47) | 0.22 (−0.07; 0.47) | 0.47 |

Urinary vanillymandelic 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; ∆Fi: percentage of heart rate deceleration; Fmin: minimum heart rate; RRmax: maximum interval between two R waves. *P ≤ 0.05; **P ≤ 0.02; ***P ≤ 0.01.

Marginal significance (0.06 ≤ P ≤ 0.10).
Figure 1. A. Correlation between Loge (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 Loge (norepinephrine) and RRmax on ECG recording during oculocardiac reflex for detecting vagal hyperreactivity, in infants with fainting spells (0.5 to 11 months old). $r = -0.25; P = 0.06$. Loge (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 $229 \pm 104$ 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 ($259 \pm 107$ nmol/mmolCr) than in infants greater or equal to 3.0 months ($179 \pm 94$ 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$_{3h}$ ($248 \pm 70$ vs $408 \pm 197$ nmol/mmolCr, $P < 0.001$). Beyond 3 months, the significance of the difference disappeared ($180 \pm 100$ vs $211 \pm 105$ 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, Log\text{\(\Delta\)}UNE\textsubscript{24h} was marginally correlated with RR\text{\(\max\)}\textsubscript{OCR} \((r = -0.25; P = 0.06)\), but not correlated significantly either with F\text{\(\min\)}\textsubscript{OCR} \((r = +0.19; P = 0.17)\) or with \(\Delta\text{F}i\text{OCR}\) \((r = -0.11; P = 0.41)\).

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

Fig. 1 shows the individual results for RR\text{\(\max\)}\textsubscript{OCR} and the correlation curve obtained. The latter shows that when RR\text{\(\max\)}\textsubscript{OCR} is greater or equal to 800 ms, Log\text{\(\Delta\)}UNE\textsubscript{24h} tends to be less or equal to 5.24 and UNE\textsubscript{24h} tends to be less or equal to 190 nmol/mmolCr.

Correlations between UNE\textsubscript{24h} and Holter ECG parameters
No correlation was found between Log\text{\(\Delta\)}UNE\textsubscript{24h} and F\text{\(\min\)}\textsubscript{HOLTER} or \(\Delta\text{F}i\text{HOLTER}\) (Table 1).

**Urinary dopamine**

Variations of excretion in function of age or collection time
For the infants of all ages, the mean UD\textsubscript{24h} = 2651 ± 1063 nmol/mmolCr (77 to 6190 nmol/mmolCr). UD\textsubscript{24h}/Cr did not vary significantly with age. UD\textsubscript{21h}/Cr was lower than UD\textsubscript{3h}/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, UD\textsubscript{24h} was not correlated with RR\text{\(\max\)} \((P = 0.90)\), with F\text{\(\min\)}\textsubscript{OCR} \((P = 0.76)\) or with \(\Delta\text{F}i\text{OCR}\) \((P = 0.47)\) (Table 1).

Correlations with Holter ECG parameters
No significant correlation was found between UD\textsubscript{24h} and F\text{\(\min\)}\textsubscript{HOLTER} \((P = 0.88)\) or \(\Delta\text{F}i\text{HOLTER}\) \((P = 0.13)\) 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.

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

U\textsubscript{24h} was also negatively correlated with VHR intensity as represented by the three OCR ECG parameters taken together and also particularly by RR\text{\(\max\)}\textsubscript{OCR} alone. If U\textsubscript{24h} is less or equal to 190 nmol/mmolCr, RR\text{\(\max\)} tends to be greater or equal to 800 ms. Low U\textsubscript{24h} 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, U\textsubscript{24h} 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 U\textsubscript{24h} and U\textsubscript{24h} 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, U\textsubscript{24h} appeared as a more promising marker than U\textsubscript{24h}. In the infants from 0.5 to 11 months, the mean LogU\textsubscript{24h} was 2.74, 2.29 and 2.20 (corresponding to U\textsubscript{24h} of
15.5, 9.9 and 9.0 nmol/mmol(Cr) 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/mmol Cr 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.

**Acknowledgements**

We thank J. Garaud, N. Mercier, the medical and paramedical clinical team of the Centre de cardiologie infantile du Château-des-Côtes, for their collaboration; Jean-Pascal Debandt and Luc Cynober for supervising the determination of catecholamines at the biochemistry laboratory of Hôtel-Dieu Hospital; J. Lellouch and J. Peyroux for useful discussion.

**Funding:** We thank Paris-6 University, “Naître et Vivre, association pour la prévention de la mort subite du nourrisson” and “Naturalia et Biologia”.

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