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Short Communication

Plasma catecholamine levels in children

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ABSTRACT

While blood norepinephrine and epinephrine levels are well established in adults, literature data concerning concentrations in young children are conflicting. This situation prompted us to assay plasma catecholamines in 86 healthy subjects aged two days to fifteen years using a sensitive high-performance liquid chromatography technique with electrochemical detection. Norepinephrine and epinephrine concentrations remained elevated up until 24 months of age, then dropped progressively to adult levels between 24 and 36 months, with fluctuations. These fluctuations, which reflect the sensitivity of children to environmental factors, prevented demonstration of any catecholaminergic secretory abnormality by blood assays in subjects under two years of age. Urinary assays, for which established values exist, are the only applicable procedure in such patients.

INTRODUCTION

While the physiopathology of the sympatho-adrenal system has been extensively investigated in newborns, few data are available for older children. Immediately after birth, the mean catecholamine concentration in the umbilical vein blood is considerably higher than in the resting adult, even in uneventful deliveries [1]. Studies on adolescents and adults have demonstrated that plasma norepinephrine concentrations increase with age [2], but few investigations have been carried out on children under two years. In order to determine whether analysis of plasma catecholamines might allow better detection of catecholaminergic secretory abnormalities than urine analysis, plasma norepinephrine (NE) and epinephrine (E) levels were investigated by sensitive high-performance liquid chromatography (HPLC) in a paediatric population without any pathology interfering with catecholamine secretion.

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EXPERIMENTAL

Subjects

The study population consisted of 86 healthy children (62 males, 24 females) aged 2 days to 15 years. All children had been born at full term (mean gestational age 39.09 ± 1.02 weeks; range 38–42 weeks); none of them had any pathology that would or was receiving any therapy that could have affected catecholamine secretion. The population was divided into six age groups: group 1 = twenty-one children aged 2–10 days; group 2 = ten children aged 10 days to 3 months; group 3 = fourteen children aged 3–12 months; group 4 = thirteen children aged 12–24 months; group 5 = eight children aged 24–36 months; group 6 = twenty children aged 3–15 years.

Samples

All blood samples were drawn from an antecubital vein at the same time of day while the children were resting in bed and as quiet as possible (07.30–08.00 h; just before the 08.00 h bottle for bottle-fed infants). Blood samples were taken shortly before discharge from the hospital, when the children had become acclimatized to the hospital environment and staff.

Reagents

Norepinephrine bitartrate, dopamine hydrochloride and alumina acid type WA-1 were obtained from Coger, and epinephrine base (E) from Helvetec (Nice, France). The internal standard (I.S.), 3,4-dihydroxybenzylamine hydrobromide (DHBA), was purchased from Aldrich and sodium octanesulphonate from Coger. All other chemicals were obtained from Merck.

Apparatus

Plasma catecholamines were measured by HPLC, with reversed-phase ion-pair chromatography and electrochemical detection with an ESA Coulochem electrochemical detector (Model 5100A, Touzart et Matignon, France): potential on D1 = 0.00 V; potential on D2 = +0.30 V. Catecholamines were separated on a 250 × 4.6 mm I.D. column of Ultrasphere ODS (average particle size 5 μm) (Beckman, Gagny, France). The mobile phase consisted of a 90:10 (v/v) mixture of 0.1 *M* sodium acetate, 0.05 *M* citric acid containing 50 mg of Na_2EDTA and 100 mg of octanesulphonic acid sodium salt per liter (pH 4.8) and methanol. It was filtered through a 0.22-μm membrane filter and degassed prior to use. A Model U6K injector was used (Water-Millipore, Saint Quentin, France).

Extraction

The extraction procedure was a modification of the technique of Mefford *et al.* [3] to use as little plasma as possible: 25 mg of alumina were added to 500 μl of plasma containing 6.25 pmol of DHBA. The pH was adjusted to 8.60 ± 0.02 with 350 μl of 1 *M* Tris–HCl buffer (pH 8.6) and the tubes were agitated for 10 min. After rapid centrifugation, the supernatant was discarded and the alumina was washed twice with water. The catecholamines were desorbed from alumina using 75 μl of 0.1 *M* perchloric acid. After 2 min of agitation, and centrifugation for 10 min at 3500 *g*, 40 μl of the supernatant were injected. A 500-μl volume of doubly distilled water spiked to obtain 5 nmol/l of each catecholamine plus 6.25 pmol of DHBA [4] was extracted in the same manner as the plasma samples. Recoveries were calculated on the basis of peak heights measured by an electronic integrator (Spectra-Physics, La Verpillière, France).

RESULTS AND DISCUSSION

The lower limits of detection were 0.20 nmol/l for NE and E; the coefficients of variation were 7 ± 2% (NE) and 9 ± 2% (E). The method separated the three main catecholamines, NE, E and dopamine (DA), but DA concentrations were below the threshold of sensitivity (0.4 nmol/l).

Fig. 1 shows the typical chromatograms of the extract of a 5 nmol/l aqueous solution of standard (A) and the extracted plasma (B) containing 3.0 nmol/l NE and 0.5 nmol/l E. DA concentration was <0.5 nmol/l.

The normal adult values established in our laboratory concur with those in the literature: NE

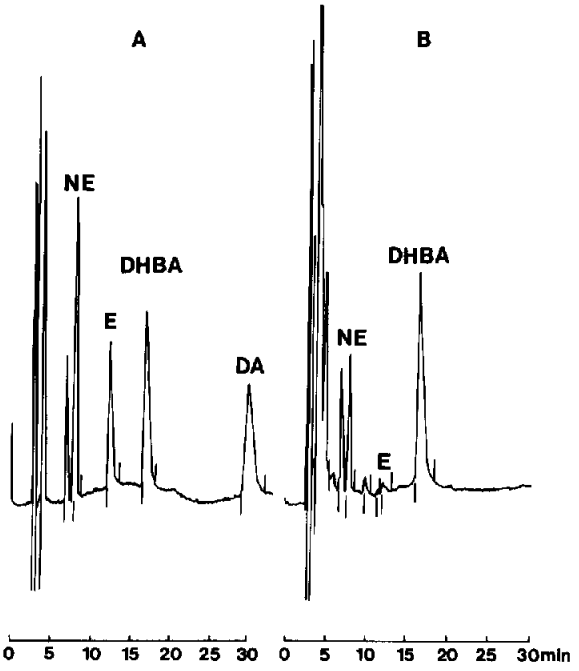

Fig. 1. Chromatograms of the extract of a 5 nmol/l aqueous solution of standard (A) and of the extract of plasma (B). NE = norepinephrine; E = epinephrine; DHBA = internal standard; DA = dopamine. In plasma, NE = 3.0 nmol/l; E = 0.5 nmol/l; DA <0.5 nmol/l (sensitivity ×4000).

below 3.1 nmol/l [5,6] and E below 0.5 nmol/l [7] under resting conditions. In this study, NE secretion rose considerably between 2 days and 12 months of age and remained high from 12 to 24 months, then decreased between 24 and 36 months with fluctuations. Table I lists the NE and E concentrations for each age group (mean, standard deviation, minimum and maximum levels) .

While E levels were moderately elevated in some children (1.75 nmol/l), they decreased after 24 months of age. The highly elevated values observed in children under 12 months decreased significantly with age, dropping to concentrations close to adult NE values at around 36 months.

During delivery, maternal plasma NE concentrations are higher than in normal non-pregnant women; the E response predominates. NE and E levels in umbilical artery blood are very high compared with maternal levels, but NE drops rapidly after birth, from 4.9 ± 0.8 nmol/l at 2 h to 1.7 ± 0.2 nmol/l at 48 h [8]. NE secretion is the predominant catecholaminergic response to parturition in the fetus and newborn [9], and circulating NE levels measured at birth are higher than in adults: 5.40 ± 2.0 nmol/l [10]. Stress scores are significantly correlated with plasma NE and E levels in neonates undergoing surgery [11], and plasma NE levels can rise significantly in response to a variety of environmental and emotional stimuli. In adults, measurement of accurate and reproducible baseline plasma cate-

TABLE I

PLASMA NE AND E LEVELS BY AGE GROUP (MEAN, S.D., MINIMUM AND MAXIMUM)

Age[a]	n	Concentration (nmol/l)							
		NE				E			
		Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.
2–10 d	21	3.5	1.74	1.0	7.6	0.85	0.68	0.2	2.5
10 d–3 m	10	5.7	3.30	2.2	13.0	0.52	0.28	0.3	1.1
3–12 m	14	3.8	1.40	1.60	6.8	0.77	0.83	0.3	2.9
12–24 m	13	3.8	3.43	0.4	11	1.11	1.17	0.2	3.9
24–36 m	8	2.8	2.94	1.1	9.8	0.63	0.87	0.1	2.7
36 m–15 y	20	2.6	2.40	0.5	10.5	0.75	0.89	0.1	3.1

[a] d = days; m = months; y = years.

cholamine levels (especially NE) requires assay in blood drawn by an indwelling venous catheter from subjects who have been at rest for at least 20 min [5]. Moreover, plasma E is known to increase when blood glucose is low [12]; this is not the case for NE. Circulating glucose levels can drop in children who have few reserves, and the moderately reduced glycaemia in the children (3.5–4.0 mmol/l) might explain why plasma E concentrations sometimes appeared moderately increased in comparison.

As observed in this study, free NE (and E) levels can be up to three times higher in healthy children under 2 years than in adults, even under true resting conditions [13]. While our results might seem to suggest that plasma NE concentration is age dependent, this is not really the case, because urinary excretion of total catecholamines is not higher in children than in adults [14]. Planz *et al.* [15] suggested that the misleading high plasma NE levels observed in very young children (under 2 years) are related to the state of agitation, because no such difference in NE levels was seen in older children under sedation. For these authors, baseline NE levels must be obtained under specific conditions of physical and psychological rest.

Overall, NE concentrations in children under 3 years, which are often higher than in adults, appear partly due to agitation. Urinary catecholamine assays should thus be performed first whenever abnormalities are suspected in young children. Diagnostic plasma catecholamine assays can be reserved for those patients in whom urinary results are inconclusive and stress can be prevented by sedation.

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REFERENCES

1 H. Lagercrantz and P. Bistoletti, *Pediatr. Res.*, 11 (1973) 889.
2 M. G. Ziegler, C. R. Lake and I. J. Kopin, *Nature*, 261 (1976) 333.
3 I. Mefford, M. M. Ward, L. Miles, B. Taylor, M. A. Chesney, D. L. Keegan and J. D. Barches, *Life Sci.*, 28 (1981) 477.
4 M. Candito, A. M. Krstulovic, V. Sbirazzuoli and P. J. Chambon, *J. Chromatogr.*, 526 (1990) 194.
5 C. R. Lake, M. G. Ziegler and I. J. Kopin, *Life Sci.*, 18 (1976) 1315.
6 P. E. Mullen, S. Lightman, C. Linsell, P. McKeon, P. S. Sever and K. Todd, *Psychoneuroendocrinology*, 6 (1981) 213.
7 P. Bouloux, D. Perret and G. M. Besser, *Ann. Clin. Biochem.*, 22 (1985) 194.
8 R. J. Eliot, R. Lam, R. D. Leake, C. J. Hobel and D. A. Fisher, *J. Pediatr.*, 90 (1980) 311.
9 A. Costa, V. De Filippis, M. Voglino, G. Giraudy, M. Massobrio, C. Benedetto, L. Marozio, M. Gallo, G. Molina and C. Fabris, *J. Endocrinol. Invest.*, 11 (1988) 703.
10 M. Sims, R. Artal, H. Quach and P. Y. Wu, *J. Perinatr. Med.*, 14 (1986) 123.
11 K. J. Anand and A. J. Aynsley-Green, *J. Pediatr. Surg.*, 128 (1988) 297.
12 O. Pryds, N. J. Christensen and B. Friis-Hansen, *Pediatrics*, 85 (1990) 172.
13 I. Eichler, H. G. Eichler, M. Rotter, P. A. Kyrle, S. Gasic and A. Korn, *Klin. Wochenschr.*, 67 (1989) 672.
14 M. Candito, A. Thyss, M. Albertini, A. Deville, S. Politano, R. Mariani and P. Chambon, *Med. Pediatr. Oncol.*, 20 (1992) 215.
15 G. Planz, J. Bicher, H. J. Mencke and G. Von Berbuth, *Experientia*, 39 (1983) 497.