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Urinary Catecholamines: Comparison Between HPLC With Electrochemical Detection and Fluorophotometric Assay

ULF LUNDBERG, LARS HOLMBERG AND MARIANNE FRANKENHAEUSER

Psychology Division, Department of Psychiatry and Psychology, Karolinska Institutet, Stockholm, Sweden

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LUNDBERG, U., L. HOLMBERG AND M. FRANKENHAEUSER. *Urinary catecholamines: Comparison between HPLC with electrochemical detection and fluorophotometric assay.* PHARMACOL BIOCHEM BEHAV 31(2) 287–289, 1988.—Comparisons between catecholamines determined by HPLC (with electrochemical detection) and fluorometry in urine samples from healthy adults and children, respectively, showed high correlations. In agreement with greater specificity for HPLC, mean values were higher for the fluorometric assay. However, it was concluded that fluorometric assays provide as valid and sensitive indices of stress-induced changes in catecholamine excretion in humans as HPLC.

Urinary catecholamines HPLC Fluorophotometric assay Human studies

THE catecholamines epinephrine and norepinephrine are sensitive indicators of psychological stress, and the development of techniques for catecholamine determination in plasma and urine has proved a useful tool in the study of human stress and coping processes over the past decades (2, 4, 5, 7, 8). The determination of catecholamines in urine is particularly useful in real life studies, where measurements are integrated for extended periods without appreciably interfering with the subject's normal habits and activities. The fluorophotometric technique [e.g., (3)] was the predominant urinary assay for several decades until more sensitive and more specific radioenzymatic (10,11) and high performance liquid chromatographic (HPLC) assays [e.g., (12)] became available.

METHOD

Material

In the present study, comparisons were made between catecholamines determined by HPLC (with electrochemical detection) and fluorometric assay of urine samples from healthy adults and children, respectively. In adults, 30 urine samples were randomly selected for this comparison from 1000 urine samples obtained from 30–50-year-old male and female white collar workers under different conditions (6). In children, 30 samples were selected randomly from 300 samples obtained from 3-year-old boys and girls during normal activities at day-care centers and at home (9). One of the urine samples from the children was lost due to technical mishap.

Catecholamine Determinations

Urine samples were collected at different times of the day 2 hours after the bladder had been emptied by voluntary voiding. For each sample, the exact time for voiding was noted, the urine volume measured and 5 ml separated for the HPLC assay. HPLC was used for the separation of the catecholamines and electrochemical detection for quantification (12). The pH of the remaining volume was adjusted to 3.0 with 2 N HCl and analyzed by the fluorimetric technique of Euler and Lishajko (3) as modified by Anderson *et al.* (1). All samples were stored at −18°C until analyzed. Values were expressed as pmol/min.

RESULTS

Figure 1 shows plots of epinephrine and norepinephrine values from HPLC and fluorimetric assays for the two sets of samples. Straight lines have been fitted to the data by the method of least squares. Table 1 shows means and standard deviations for each set of data and product moment correlations between the methods. Analysis of variance was used to test the difference between means.

Figure 1 and Table 1 show that there was good agreement between the two methods. There was no marked deviation from linearity. The higher correlations in adults as compared with the children are probably related to their higher mean values and greater range. In the adult sample, HPLC gave significantly ($p<0.0001$) lower values for both catecholamines than the fluorometric assay. In the children's sample, one epinephrine HPLC assay (see upper right hand diagram

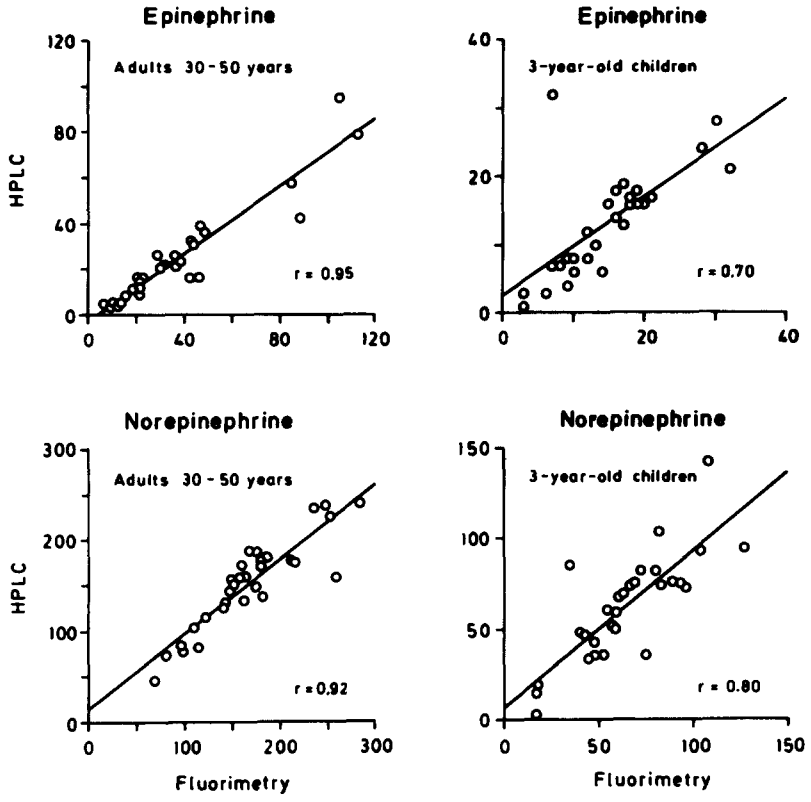

FIG. 1. Catacholamine values (pmol/min) obtained by HPLC plotted against values from fluorometric determination of epinephrine and norepinephrine in urine samples from adults (30–50 years) and children (3 years), respectively.

of Fig. 1) deviated markedly from the rest, thus affecting the slope of the line fitted to the data. After exclusion of this value, the correlation between methods for epinephrine in children increased from .70 to .93 and the ANOVA between means was highly significant ($p<0.0001$) for epinephrine as in the adults' sample, but not for norepinephrine.

The results showed high correlations between urinary catecholamines determined by HPLC with electrochemical detection and by fluorometric assay. However, the fluorometric assay gave consistently higher values. In agreement with greater specificity for HPLC, this indicates that the fluorometric method tends to overestimate the amount of free catecholamines in the urine. This systematic difference in absolute values does, however, not affect the use of urinary catecholamines as indices of changes in stress level induced by environmental conditions, nor does it affect comparisons between groups.

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TABLE 1

MEANS AND STANDARD DEVIATIONS FOR URINARY EPINEPHRINE AND NOREPINEPHRINE (pmol/min) DETERMINED BY HPLC WITH ELECTROCHEMICAL DETECTION AND FLUOROPHOTOMETRIC ASSAY, RESPECTIVELY, IN SAMPLES FROM ADULTS AND CHILDREN, RESPECTIVELY

	Adults (30–50 years)		Children (3 years)	
	HPLC	Fluoro-metry	HPLC	Fluoro-metry
Epinephrine (pmol/min)				
Mean	23.9	36.4	12.6	15.1
SD	21.6	27.7	6.8	7.3
n	30	30	28	28
ANOVA of means				
F	47.5		22.8	
df	1/29		1/27	
p	<0.0001		<0.0001	
Product moment correlation coefficient	.95*		.93*	
Norepinephrine (pmol/min)				
Mean	155.6	172.0	62.3	64.1
SD	53.0	59.1	29.3	27.2
n	30	30	29	29
ANOVA of means				
F	14.6		0.38	
df	1/29		1/28	
p	<0.001		n.s.	
Product moment correlation coefficient	.92*		.80*	

*$p<0.001$.

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