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Urinary Free and Conjugated Catecholamines and Metabolites in Autistic Children[1]

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Urinary catecholamines (DA, NE, E) and their main metabolites (HVA, DOPAC, MHPG) were analyzed both as free and conjugates in eight children diagnosed as autistic according to DSM-III criteria and eight normal children. Significant differences appeared for the urinary excretion of both DA and NE and their respective metabolites: Autistic children showed low DA, high HVA, high NE, low MHPG urinary levels. These results are consistent with previous findings on altered catecholamine metabolism in autistic children. They suggest that autistic behaviors might be related to an abnormal functional imbalance among monoamines either at a molecular level or at a system level. Furthermore, they emphasize the special interest of urinary assays in pediatric research.

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INTRODUCTION

Clinical, pharmacological, and biochemical studies suggest a possible involvement of brain dopamine (DA) and norepinephrine (NE) in the production of autistic symptoms. In search of some neurochemical correlates of infantile autism, various authors tested these catecholamine-related hypotheses (Young, Cohen, et al., 1982; Young, Kavanagh, Anderson, Shaywitz, & Cohen, 1982). Several observations suggest that autism could be associated with a central dopaminergic hyperactivity. Neuroleptic agents—DA-receptor blockers—significantly reduce behavioral symptoms such as stereotypies and withdrawal and they facilitate learning (Anderson et al., 1984; Campbell et al., 1982). Dextro and levo-amphetamine—indirect DA agonists—exacerbate autistic symptoms in most cases (Campbell et al., 1976). Changes in catecholaminergic activity are known to be reflected in the content of their major metabolites in cerebrospinal and peripheral fluids. High levels of homovanillic acid (HVA, the principal DA metabolite) have been found in the cerebrospinal fluid (CSF) of several impaired autistic children (Cohen, Caparulo, Shaywitz, & Bowers, 1977; Cohen, Shaywitz, Johnson, & Bowers, 1974; Gillberg, Svennerholm, & Hamilton-Hellberg, 1983); Lelord et al. (1978) reported that urinary HVA levels were higher in autistic than in normal children. Results from studies on NE metabolism may appear heterogeneous. It was reported that plasma NE levels were elevated in autistic patients in both the lying and standing positions (Lake, Ziegler, & Murphy, 1977). In contrast, a decrease of urinary free catecholamines and 3-methoxy-4-hydroxy-phenyl glycol (MHPG, the principal brain metabolite of NE) was found in autistic children (Young, Cohen, Brown, & Caparulo, 1978; Young, Cohen, Caparulo, Brown, & Maas, 1979).

The hypothesis of an alteration of central catecholaminergic function in autistic children needs to be clarified. However, several methodological problems have to be overcome: the need for venous or lumbar puncture in children is one of the main limiting difficulties.

CSF cannot be obtained for study from normal children. This is why, in most of the previous studies, it has been necessary to contrast one diagnostic group with another and to use autistic and other neuropsychiatrically disabled children with various neurological disorders (Shaywitz, Cohen, Leckman, Young, & Bowers, 1980; Young et al., 1981).

Part of these ethical and methodological problems could be overcome through the use of urinary assays. Because of the variety of adrenergic metabolites, suitable information on peripheral or central catecholamine function may be obtained from urinary studies. Indeed, in contrast to urinary catecholamines (E, NE, DA) which are well-known indexes of sympathoadrenal activity, several lines of evidence indicate that deaminated metabolites (MHPG, HVA, and DOPAC) might derive in part from CNS

metabolism (Shekim, Javaid, Davis, & Bylund, 1983). Using arterio-venous determinations, Maas, Hattox, Green, and Landis (1979), Maas, Hattox, Martin, and Landis (1979), and Swann, Maas, Hattox, and Landis (1980) were able to demonstrate the HVA and MHPG output from brain into plasma and calculated that at least 50% of urine total MHPG and 33% of urine free HVA derived from CNS metabolism in man. A positive correlation was found between plasma free and urinary total MHPG levels (Young et al., 1981). The hypothesis of an independent (peripheral or central) origin of free, sulfate, and glycuronide MHPG present in human urine was examined by Peyrin and Pequignot (1983). According to their results, the sympathetic nervous system seems to be the main source of glycuronide and arguments are given supporting the central origin of sulfate. According to Kopin (1985), the measurement of plasma levels or urinary excretion of MHPG does not appear to be a valid index of brain NE metabolism. However, the observations showing differences among patient groups remain valid. A correspondence between CSF, plasma, and urinary HVA levels has not been yet clearly established in man. If CSF HVA is a good index of DA metabolism in the CNS, the measurement of HVA in plasma or urine reflects formation of the metabolite in peripheral tissues as well as in the brain (Kopin, 1985). In animal studies, urinary excretion of free and conjugated DA metabolites (HVA, DOPAC: 3, 4-dihydroxyphenylacetic acid) appears to be modified after lesions of dopaminergic pathways (Peyrin, Simon, Cottet-Emard, Bruneau, & Le Moal, 1982). In man, a negative correlation was found between age and urinary HVA levels (Dalmaz, Peyrin, Sann, & Dutruge, 1979; Garreau et al., 1981).

The aim of the present study was to determine whether systematic differences in urinary catecholamine excretion existed between two groups of children. Autistic patients recruited on the basis of careful diagnosis criteria were compared to normal children. A wide range of urinary compounds could be simultaneously investigated: DA, NE, E, and their main metabolites (HVA, DOPAC, MHPG) were analyzed both as free and conjugated. In addition, urinary free and conjugated DOPA was determined.

METHOD

Subjects

Two groups of children, sex- and age-matched, were recruited for this study: eight normal children living in their families (5 boys aged from 3 to 10, mean age 6.2 years) and eight autistic children (aged from 4.4 to 8.3, mean age 5.7 years). The autistic subjects were selected from a group of 40 autistic inpatients of our Child Psychiatry Unit and considered as present-

ing the full typical syndrome of autism. The diagnosis of infantile autism was made by two research child psychiatrists on the basis of DSM-III (American Psychiatric Association, 1980) criteria. The children received a complete diagnostic work up including medical, neurological, psychiatric, and psychological evaluation. Patients with gross neurological deficits were excluded from the study. Two of the eight autistic children were diagnosed as having epilepsy, two others had EEG abnormalities. Two children had little or no language, and six had some useful speech albeit with abnormalities such as echolalia. Six of these eight children were in the mildly–moderately mentally retarded range and two were severely mentally retarded. Three children were generally hyperactive, whereas the remaining were considered normo- or hypo-active (Table I). None received antipsychotic medication.

Biochemical Determination

After 24-hour tyrosine dietary restriction, urine samples were collected on a daily period of 7 hours (from 9 a.m. to 4 p.m.) after elimination of nighttime urines. The collection was performed in exactly the same conditions at home for normal children and at the hospital for autistic patients.

Urines were acidified ($pH \leq 4$ and frozen at $-18C$. Catecholamines (E, NE, DA) and DOPAC were extracted from urine using a double-step ion-exchange method and assayed by automated procedures (Cottet-Emard & Peyrin, 1982; Lelord, Callaway, & Muh, 1982). Two aliquots (10 ml) were taken from each urine sample and analyzed, respectively, for their free and total content of catecholamines and DOPAC. The amount of the conjugated form was calculated by the difference between the total and free estimation. Free, sulfate, and glycuronide MHPG were determined separately in three urine aliquots (3 ml) as previously described (Lelord et al., 1982; Peyrin & Pequignot, 1983). Free and total HVA were determined in two 10-ml aliquots. Samples were extracted by ethylacetate and HVA was then assayed by gas chromatography, according to a method adapted from Roginski, Gordon, and Bennett (1974) and Wadman, Ketting, and Voute (1976).

Creatinine was assayed by automated colorimetry (Paget, Gontier, & Liefooghe, 1955). Autistic and normal group values for all clinical and biochemical parameters were compared using the Mann-Whitney U test.

RESULTS

Physical Parameters. As shown in Table II, no significant differences occurred between autistic and normal group means for height, head circumference, and weight.

Table I. Characteristics of the Autistic Group

Case no.	Sex	Neurological examination	Language	IQ	Activity
1	F	Normal	Present	50–70	Hyper
2	M	Normal	Present	50–70	Normal
3	M	Normal	Present	35–49	Normal
4	F	EEG abnormal	Present	50–70	Normal
5	M	Epilepsy	Present	35–79	Hyper
6	F	Normal	Present	50–70	Normal
7	M	EEG abnormal	Absent	20–34	Normal
8	M	Epilepsy	Absent	20–34	Hyper

Table II. Comparison of Ages and Physical Parameters Between the Autistic and Normal Groups

Group	Age (years)	Height (cm)	Head (cm)	Weight (kg)
Autistic	5.8 ± 0.5	106.3 ± 3.1	50.3 ± 0.9	17.7 ± 1.5
Normal	6.22 ± 0.98	115 ± 5.33	51.44 ± 0.55	22.7 ± 2.3

Urinary Compounds (Table III and Figure 1). Mean creatinine concentrations did not differ significantly when autistic and control groups were compared (Autistic: 564.9 ± 83.9 mg/L; Normal: 640.5 ± 96.6 mg/L; ns).

DOPA and Catecholamines. In contrast to E excretion, which was in the normal range, free and total NE levels were higher in the autistic than in the control group. Low levels of DOPA (conjugated) and dopamine (conjugated and total) were found in the autistic group.

Deaminated Metabolites. In autistic children, free and total MHPG were slightly decreased, free HVA was slightly increased while conjugated DOPAC was significantly decreased.

DISCUSSION

These preliminary results obtained in the comparison of autistic and normal children show differences in the urinary excretion of DA, NE, and their respective metabolites: The autistic children showed increased HVA levels while their DA and DOPAC levels were decreased. In fact, the results concerning DOPAC must be interpreted cautiously since the DOPAC in our controls is unusually high (Kopin, 1985; Zametkin et al., 1985). Dietary factors might have influenced our results. The pattern was reversed for MHPG and NE levels: low MHPG and high NE levels. The decreased MHPG is consistent with previous findings (Young et al., 1979) but the increased NE is not in keeping with usual patterns, and thus difficult to interpret.

Table III. Urinary Excretion of DOPA, DA, NE, E, and Their Metabolites in Normal and Autistic Children[a]

	Normal	Autistic
DOPA		
Free	80.25 ± 5.32	74.63 ± 14.34
Conjugated	36.50 ± 8.14	12.50 ± 4.64[c]
Total	116.75 ± 8.49	86.75 ± 14.89
DA		
Free	521.13 ± 121.57	294.63 ± 30.89
Conjugated	704.50 ± 175.65	282.75 ± 69.41[b]
Total	1,225.63 ± 143.88	577.38 ± 49.87[e]
DOPAC		
Free	1,050 ± 160	1,150 ± 270
Conjugated	7,280 ± 1,720	2,040 ± 530[d]
Total	8,450 ± 1,820	3,370 ± 680
HVA		
Free	5,710 ± 900	9,760 ± 1,710[b]
Conjugated	2,820 ± 870	3,380 ± 920
Total	8,490 ± 1,440	13,330 ± 2,110
NE		
Free	27.44 ± 6.24	66.13 ± 10.96[d]
Conjugated	67.06 ± 10.43	93.13 ± 19.70
Total	93.00 ± 12.93	159.25 ± 18.33[d]
E		
Free	8.63 ± 2.58	9.01 ± 0.85
Conjugated	6.90 ± 2.27	11.38 ± 2.67
Total	15.52 ± 2.91	20.38 ± 3.36
MHPG		
Free	320 ± 110	100 ± 20[c]
Conjugated sulfate	1,180 ± 150	1,530 ± 680
Conjugated glycuronide	530 ± 70	490 ± 50
Total	2,040 ± 200	1,410 ± 100[b]

[a]3, 4-Dyhydroxyphenylalanine, DOPA; dopamine, DA; 3, 4-dihydroxyphenylacetic acid, DOPAC; homovanillic acid, HVA; norepinephrine, NE; epinephrine, E; 3-methoxy-4-hydroxy-phenylglycol, MHPG. Mean ± *SE*; ng/mg creatinine.
[b]$p < .05$, Mann Whitney U test.
[c]$p < .02$, Mann Whitney U test.
[d]$p < .01$, Mann Whitney U test.
[e]$p < .001$, Mann Whitney U test.

Our data were not related to an age or growth difference between groups and urinary creatinine concentration in the autistic and control group were similar. The values obtained for DA, NE, and E (ng/mg creatinine) with a 7-hour collection are similar to those previously obtained with 24-hour urines in normal children of same age (Dalmaz & Peyrin, 1982).

Most of our findings are consistent with some previous reports suggesting that autistic behaviors might be related to an abnormal functional

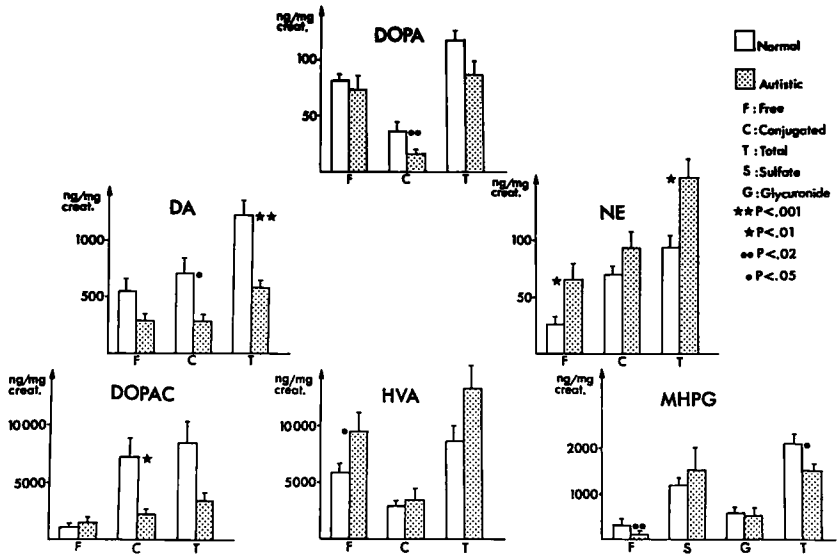

Fig. 1. Mean urinary levels (ng/mg creatinine) of DOPA, dopamine (DA), norepinephrine (NE), 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) in normal and autistic children.

imbalance among monoamines either at a molecular level or at a system level (Garnier et al., 1986; Gillberg et al., 1983; Young, Cohen, et al., 1982; Young, Kavanagh, et al., 1982). However such results must be interpreted cautiously because of the small number of patients in each group. Additional studies which might confirm such differences between groups are necessary.

In future studies it would be of great interest to investigate the urinary excretion of catecholamines and serotonin together in carefully defined subgroups of autistic retarded and normal children. Group differences with regard to clinical features (especially sex, motor symptoms such as hyperactivity, and stereotypies) and biochemical data could be thus precisely analyzed.

Finally, it is worth mentioning the special usefulness of urinary assays in pediatric research. Because they are atraumatic, such examinations can be repeated and thus provide helpful indications for the evaluation in therapeutic studies.

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