Homovanillic Acid Content of Neuroblastomas* (33700)

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The compound 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA), normally present in urine, is excreted in marked excess in the urine of most patients with neuroblastoma (1, 2). Levels of the precursors of HVA, 3,-4-dihydroxyphenylalanine (DOPA) and 3,-4-dihydroxyphenylethylamine (dopamine) are also elevated in the urine of most of these patients. HVA is produced from dopamine by an enzymatic transformation involving catechol-*O*-methyl transferase (COMT) and monoamine oxidase (MAO). In normal individuals most dopamine is converted to norepinephrine (NE) or epinephrine (E), which are subsequently degraded enzymatically by COMT and MAO to form 3-methoxy-4-hydroxymandelic acid and other catabolities (VMA).

It is not clear whether the excessive HVA found in the urine of patients with neuroblastoma is produced in the liver and kidney, tissues known to have high activities of COMT and MAO, or in the tumor tissue itself (3, 4). In order to investigate this problem, we recently analyzed a number of neuroblastomas for concentration of HVA. The findings were correlated with urinary excretion of HVA and other catecholamine metabolites.

Materials and Methods. The concentration of HVA in tumor tissue was measured by a modification of the method of Anden *et al.* (5). Approximately 1 g of tissue was homogenized in a Waring blendor with 0.1 *N* hydrochloric acid. Protein was precipitated with zinc sulfate and sodium hydroxide. The HVA-containing supernatant was washed with chloroform, acidified, saturated with sodium chloride, and extracted with ether. The ether extract was immersed in an ethanol–dry ice bath for 1 hr, filtered, brought to room temperature, and the HVA was extracted in 0.05 *M* Tris buffer at pH 7.5. The HVA was prepared for fluorometric assay by adjustment of the pH to above 7 with 5 *N* ammonium hydroxide and oxidation with 0.01% potassium ferricyanide. Oxidation was stopped after 4 min with 0.1% L-cysteine, and the samples were read in an Aminco-Bowman spectrophotofluorometer at 320 mμ activation and 425 mμ emission wavelengths. HVA standards, diluted to the same buffer strength as the tumor samples, were carried through the entire procedure and included in every determination.

Urinary HVA was determined by the method of Sato (6). Urinary and tissue E and NE were measured by a modification of the trihydroxyindole reaction using iodine as the oxidant (7). Urinary and tissue metanephrine (MN) and normetanephrine (NMN) were measured by the methods of Pisano (8) and Crout *et al.* (9). VMA was determined by the method of Pisano *et al.* (10).

Results. The concentration of HVA was determined in 12 neuroblastomas. Clinical data is presented in Table I. HVA was detected in 9 of the 12 tumors, with concentrations varying from 0.4 to 11.0 μ/g of tissue (Table II). In one case, no. 9, both primary tumor and metastatic tumor tissue were analyzed, and both were found to have similar concentrations of HVA. In another case, no. 8, a biopsy specimen was analyzed when the diagnosis was first made, and an autopsy sample from the primary tumor was analyzed 1 year later. The concentration of HVA in the autopsy specimen was almost seven times that found in the biopsy.

The urinary excretion of HVA, VMA, NMN and MN, and NE and E for 9 of the cases is also presented in Table II. In 7 of these 9 cases detectable amounts of HVA were found in the tumor tissue. In 2 cases with no detectable tissue HVA, one patient

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TABLE I. Patient Data from 12 Cases of Neuroblastoma.

Patient	Age	Sex	Specimen	Tumor wt. (g)
1. I.M.	—	—	Surgical	—
2. S.S.	10 mo	M	Autopsy	122
3. D.L.	4 yr	F	Surgical	95
4. C.T.	1 yr	M	Surgical	775
5. R.K.	—	—	Surgical	Biopsy
6. J.W.	2 yr	F	Surgical	40
7. D.S.	—	M	Surgical	50
8a. L.W.	2 yr	M	Surgical	Biopsy
b. L.W.	2 yr	M	Autopsy	800
9a. D.S.	4 yr	M	Surgical[a]	—
b. D.S.	4 yr	M	Surgical[b]	—
10. W.G.	17 mo	M	Surgical	36
11. A.	—	—	Surgical	—
12. B.M.	15 yr	F	Surgical	Biopsy

[a] Primary tumor.
[b] Metastasis.

had a low excretion of urinary HVA and the other had an abnormally high excretion. Tumor tissue concentrations of NMN and MN and NE and E were determined and are also presented in Table II.

A Kendall's rank correlation test (11) was performed on all the data in Table II. The results are shown in Table III. Significant correlation was found only between tumor tissue and urine concentration of HVA.

One ganglioneuroma, one benign pheochromocytoma, and one malignant pheochromocytoma were analyzed for tissue concentration of HVA (Table IV). A concentration of 0.3 μg/g was found in the malignant pheochromocytoma, a level somewhat below that present in most of the neuroblastomas. HVA was not detected in the benign pheochromocytoma or in the ganglioneuroma.

TABLE III. Statistical Correlation of Tumor HVA Concentration and Other Tumor or Urinary Catecholamine Concentrations.[a]

Tumor HVA vs	N	T	p
Tumor NMN + MN	10	0.0667	0.398
Tumor NE + E	10	−0.3755	0.111
Urine VMA	9	0.1667	0.267
Urine NE + E	9	−0.1667	0.267
Urine NMN + MN	9	0.2777	0.145
Urine HVA	11	0.5272	0.011

[a] Where N = number of pairs of observations; T = Kendall's rank correlation coefficient.

Specimens of tissue from the adrenal glands, kidney, spleen, liver, heart, and lung were obtained from three adults at autopsy and analyzed for their HVA concentration.

TABLE II. Tumor Concentration and Urinary Excretion of Catecholamines in Neuroblastoma Patients.

	Tumor conc (μg/g)			Urinary excretion (mg/24 hr)			
Case	HVA	NE and E	NMN and MN	HVA	VMA	NE and E	NMN and MN
1.	1.8	1.62	1.54	—	—	—	—
2.	2.6	13.7	5.1	14.1	21.4	0.009	1.45
3.	3.0	21.7	0.9	33.6	7.6	0.048	1.40
4.	0.8	0.56	11.1	33.6	6.5	0.218	1.40
5.	1.0	17.4	0.0	37.0	33.0	0.042	9.70
6.	0.4	31.2	4.1	7.1	2.7	0.074	1.74
7.	4.1	53.0	0.39	—	—	—	—
8a.	1.6	—	—	49.0	6.6	0.029	0.63
b.	11.0	0.20	0.0	61.7	3.4	0.052	0.63
9a.	2.1	32.1	8.6	52.5	51.9	0.050	17.8
b.	1.7	17.5	4.7	52.5	51.9	0.050	17.8
10.	0.0	0.29	0.0	17.9	13.7	0.27	2.05
11.	0.0	0.0	0.63	—	—	—	—
12.	0.0	23.9	7.2	2.0	9.0	0.078	1.95
Adult normals	—	—	—	10.0	5.0	0.06	1.0

TABLE IV. Concentration of HVA and Other Catecholamines in Tissue and Urine of Other Neural Crest Tumor Patients.

History		Tumor	Conc (μg/g of tissue)		Urine			
Case	Diagnosis	HVA	NE + E	NMN + MN	HVA	VMA	NE+E	NMN + MN
1. W.	Ganglioneuroma	0.0	0.61	0.63	—	—	—	—
2. TB.	Pheo—benign	0.0	2740	17.1	—	—	—	—
3. MW.	Pheo—malig.	0.3	10,550	77.2	1.39	42.2	2.07	19.5

No HVA was detected in samples from any of the tissues except those of the kidney and adrenal gland. The small amount found in the kidney is believed to be an artifact resulting from the very high blank values obtained. The adrenal gland contained an average of 0.3 μg/g of tissue.

Discussion. This study demonstrates that detectable amounts of HVA can be found in most neuroblastomas. These findings indicate that dopamine can be degraded in the tumor tissue to HVA in the presence of COMT and MAO. Voorhees (12) has previously found by paper chromatography detectable amounts of HVA in 2 of 5 neuroblastomas and 4 of 5 ganglioneuromas. The levels of HVA found are relatively low and approximately in the same range as those reported for corpus striatum in rabbits by Sharman (13) and humans by Bernheimer (14). The low level of HVA indicates either low production from dopamine or more likely rapid turnover and secretion of HVA by tumor tissue. Increased urinary excretion of HVA was demonstrated in one patient with no detectable levels of HVA in the tumor. This suggests that dopamine, in this case, was converted to HVA in tissues other than the tumor, possibly in liver, adrenal, and kidney tissue, known to contain large quantities of MAO and COMT activity. Analysis of the data indicates that the increased urinary excretion of HVA found in cases of neuroblastoma may result from the formation of this compound in both tumor tissue and nontumor tissue.

There are significant differences in catecholamine metabolism between neurozlastomas and benign pheochromocytomas (1, 2). In most neuroblastomas there is low storage and rapid turnover of NE, incomplete conversion of dopamine to NE, and some conversion of dopamine to HVA within the tumors. In benign pheochromocytomas, on the other hand, almost complete conversion of dopamine to NE and E occurs with greater storage of NE and E.

In the storage of NE and E, malignant pheochromocytomas resemble benign pheochromocytomas. In many of the maligant tumors, however, there is increased urinary secretion of dopamine and HVA (15). The finding in the malignant pheochromocytoma analyzed in this study indicates that conversion of dopamine to HVA can occur in malignant pheochromocytoma tissue.

Adequate biochemical explanation for the metabolic differences between these tumors will require additional study. It does appear, however, that in these various tumors there may be significant differences in the intracellular binding of dopamine. These differences may be reflected in part by the secretion of dopamine by most neuroblastomas but not by benign pheochromocytomas. The apparent availability of dopamine for degradation to HVA in neuroblastomas but not benign pheochromocytomas may also reflect differences in the intracellular compartmentalization of dopamine.

Summary. Tumor tissues from 12 neuroblastomas, one benign pheochromocytoma, one malignant pheochromocytoma, and one ganglioneuroma were analyzed for HVA. Autopsy specimens of heart, lung, liver, spleen, kidney, and adrenal gland were analyzed also. The compound was detected in 9 of the neuroblastomas and in the malignant pheochromocytoma. A small amount was detected in adrenal tissue.

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Divalent Cation Requirements for Leukocyte Adhesiveness* (33701)

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Both polymorphonuclear leukocyte (PMN) aggregation and PMN adhesion to glass are increased after phagocytosis of bacteria or exposure to bacterial endotoxin (1). The PMN adhesiveness is abolished by chelation with EDTA and is considered to be a divalent cation-dependent process (2, 3). A significant disadvantage of chelation studies with EDTA is that this agent binds calcium and magnesium with approximately the same avidity and, therefore, cannot be used to differentiate the effects of calcium and magnesium ion on cell function. Moreover, the magnesium salt of EDTA cannot be used to differentiate calcium ion and magnesium ion effects because plasma containing magnesium EDTA has appreciable concentrations of calcium ion (4). In contrast, the magnesium salt of ethylene glycol diamino ethyl tetraacetic acid (EGTA) is an effective agent for distinguishing between Mg^{2+} and Ca^{2+} requirements since EGTA binds calcium ion 100,000-fold more avidly than magnesium ion (4). Plasma containing MgEGTA has a slight excess of magnesium ion and is virtually free of calcium ion.

In the present study the effects of the sodium and the magnesium salts of EDTA and EGTA on PMN adhesion to glass and on human PMN aggregation after exposure to endotoxin were determined.

Methods. Blood from normal human donors was heparinized with 4 mg/100 ml of preservative-free heparin.[2] Chelating agents were added to blood to achieve 10 m*M* concentration of various salts of EDTA[3] or EGTA.[4] Equal volumes of saline were added to control specimens.

Techniques for determination of leukocyte adhesion were previously described (5). In brief, leukocytes in horizontally placed capillary tubes were allowed to sediment to the side for 1 hr at 37°. The tubes were then centrifuged at 16,000 rpm for 10 sec and the number of leukocytes remaining adherent to

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