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Plasma Catechols in Familial Dysautonomia: A Long-term Follow-up Study

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Abstract

This study tested whether familial dysautonomia (FD) involves progressive loss of noradrenergic nerves. Plasma levels of catechols, including dihydroxyphenylglycol (DHPG), norepinephrine (NE), dopamine (DA), and DOPA, were measured in 7 adult patients with FD and 50 healthy control subjects. FD patients were re-tested after a mean follow-up period of 13 years. Compared to controls, FD patients had low plasma levels of DHPG ($P < 0.001$), high DOPA and DA levels ($P = 0.01$, $P = 0.0002$), and high NE:DHPG ($P < 0.0001$), DA:NE ($P = 0.0003$), and DOPA:DHPG ($P < 0.0001$) ratios. At follow-up there were no changes in plasma levels of individual catechols; however, there were further increases in DOPA:DHPG ratios (mean $24 \pm 7\%$, $P = 0.01$). In FD, plasma catechol profiles are sufficiently stable, at least over a decade, to be used as a biomarker of disease involvement. An increasing DOPA:DHPG ratio suggests slight but consistent, progressive loss of noradrenergic neurons.

Keywords

Familial dysautonomia; Dihydroxyphenylglycol; Norepinephrine; DOPA; Sympathetic nervous system

Introduction

Familial dysautonomia (FD), also known as Riley-Day syndrome and hereditary sensory and autonomic neuropathy type III, is a rare inherited disorder, transmitted as an autosomal recessive trait in individuals of Ashkenazic extraction. FD features extensive sensory and autonomic dysfunction. The etiologic basis is mutation of the gene, *IKBKAP*. The most common mutation causes a splicing alteration that leads to tissue-specific decreased

expression of IkappaB kinase-associated protein—especially in neuronal tissue [1]. This protein, now termed ELP-1, is a member of the human Elongator complex, which aids in transcription. As a result of decreased functional Elongator, several other target genes are down-regulated [2]. Many of the down-regulated genes participate in cell migration and survival, and this may explain neuropathological findings such as diminished numbers of small nerve fibers and neuronal loss in dorsal root ganglia [3]. The decrease in sympathetic neuronal innervation in FD seems to be pervasive, involving the skin [4], renal blood vessels [5], and the heart (unpublished observations).

Consistent with diminished sympathetic cardiovascular innervation, patients with FD have prominent orthostatic hypotension, which is associated with deficient orthostatic increments in plasma levels of the sympathetic neuro-transmitter, norepinephrine (noradrenaline, NE) [6]. Plasma NE levels are normal, however, when the patients are resting supine. Urinary excretion rates of dopamine (DA) and its metabolites are increased relative to those of NE and its metabolites [7, 8], findings that might indicate decreased activity of DA-beta-hydroxylase, the enzyme that catalyzes conversion of DA to NE. One report noted low plasma levels of DA-beta-hydroxylase activity in FD [9]; however, a subsequent study failed to confirm this finding [10]. It is by now accepted that neurochemical data consistent with decreased DA-beta-hydroxylase activity in FD actually reflect decreased numbers of sympathetic noradrenergic neurons that contain DA-beta-hydroxylase, not deficiency of the enzyme per se.

In addition to attenuated orthostatic increments in plasma levels of NE, FD patients have a high ratio of 3,4-dihydroxyphenylalanine (DOPA) to dihydroxyphenylglycol (DHPG) in plasma [11]. Since DOPA is the immediate product of tyrosine hydroxylation, the enzymatic rate-limiting step in catecholamine synthesis, and DHPG is an index of norepinephrine turnover [12], the elevated DOPA: DHPG ratio may result from compensatorily increased NE production in a smaller complement of sympathetic nerves, in line with the post-mortem histopathologic finding of increased tyrosine hydroxylase immunoreactivity in sympathetic ganglionic neurons [13].

FD involves not only impaired development of unmyelinated and small myelinated neurons initially but also progressive loss of sensory neurons, as demonstrated by increased early appearance of residual nodules in dorsal root ganglia. Progressive sensory dysfunction has been demonstrated using clinical measures [14]. Whether there is also progressive loss of sympathetic nerves has been unknown. Based on the hypothesis that such progression would be associated with worse catechol profiles, we performed a long-term follow-up study in adult patients with FD, to determine if there were individual as well as group changes over time that would provide neurochemical means of assessing progression of disease and responses to treatment.

Methods

Seven FD patients (3 males, 4 females, mean age $25 \pm$ (SEM) 3 years at the time of initial testing) were studied at the Dysautonomia Treatment and Evaluation Center at New York University Medical Center. All met clinical diagnostic criteria for FD, as follows. They were

of Ashkenazi Jewish extraction, lacked lingual fungiform papillae, did not have an axon flare after intradermal histamine injection, had decreased or absent deep tendon reflexes, and lacked overflow tears. In addition, all proved to be homozygous for the missplicing *IKBKAP* mutation. The patients were relatively stable and ambulatory, although the oldest FD subject had severe ataxia and required a walker at the time of follow-up testing. Each patient had two testing sessions, separated by a mean of 13 years (age at follow-up 38 ± 2 years). Initial results from the 7 FD patients were reported previously as part of a group of 10 patients [11]. Of the original 10 patients, 2 males were deceased and 1 female lived outside the greater New York area.

All subjects in this study gave written informed consent to participate in protocols approved by the Institutional Review Board of New York University Medical Center or by the National Institute of Neurological Disorders and Stroke. Plasma catechol data from the FD patients were compared to those in a database of 50 age-matched healthy control subjects tested in the Clinical Neurocardiology Section.

Familial dysautonomia patients were instructed to stop all medications, including antihistamines and caffeine, for 24 h prior to each testing session. None was on a medication known to interfere with neuronal uptake of catecholamines.

Blood was sampled via an indwelling arm venous catheter or butterfly needle, after the subject was at supine rest for at least 15 min. After refrigerated centrifugation, the plasma was transferred to plastic cryotubes and stored at -70 degrees centigrade or colder until assayed. Plasma levels of catechols were assayed in the Clinical Neuro-chemistry Laboratory of the Clinical Neurocardiology Section, as described previously [15]. Briefly, the catechols were purified partially by batch alumina extraction, separated by high pressure liquid chromatography, and quantified by the current produced upon exposure of the effluent to a series of oxidizing and then reducing potentials.

Absolute values for plasma levels of catechols were log transformed, to achieve homogeneity of variance. Mean values for plasma catechols were compared between the FD and the control group by independent-means t-tests, and changes between initial and follow-up testing were compared in FD patients by paired t-tests. Frequency data in the FD and control groups were compared by calculation of χ^2 . All mean values were expressed $\pm$ SEM. A *P*-value less than 0.05 defined statistical significance.

Results

Both at the time of initial testing and upon follow-up testing, the FD group had higher mean DA and DOPA levels and markedly lower mean DHPG levels than did the control group (Table 1). The groups did not differ significantly in mean NE, epinephrine (EPI), or dihydroxyphenylacetic acid (DOPAC) levels.

At both testing times, the FD group also had higher mean DA:NE, NE:DHPG, DOPA:DHPG, and DOPAC: DHPG ratios than did the control group (Table 1). All the FD patients had a DOPA:DHPG ratio more than 2.8 and DA:NE ratio more than 0.06, both initially and at follow-up, a combination not found in any control subject ($\chi^2 = 194$, $P <$

10^{-10}), and all the FD patients had DOPA:DHPG ratios that were more than 2 standard deviations above the control mean at both testing times (Fig. 1). Across all subjects and testing times, DOPA:DHPG ratios were

Over the 13 years of follow-up, there were no significant further changes in plasma levels of any of the catechols. Values for ratios of plasma catechols also did not change significantly, except that all FD patients had an increase in the DOPA:DHPG ratio (mean $24 \pm 7\%$, $P = 0.01$; Fig. 2).

Discussion

The clinical neurochemical results of the present study enabled testing of the hypothesis that FD involves both arrested development of sympathetic noradrenergic nerves and chronic, progressive loss of those nerves in adult life. In general, whereas the data provide clear support for arrested development, evidence for further loss is subtle, possibly because of compensatory increases in catecholamine biosynthesis and release.

To interpret the clinical neurochemical findings requires understanding of the sources and meanings of plasma levels of the endogenous catechols, which are the catecholamines (NE, EPI, and DA), the precursor of the catecholamines (DOPA), the main neuronal metabolite of NE (DHPG), and the deaminated metabolite of DA (DOPAC) [16].

Plasma levels of NE mainly reflect exocytotic release, neuronal and extra-neuronal uptake, and renal filtration and secretion. Accordingly, plasma NE is related only distantly and complexly to sympathetic innervation density. Plasma DHPG is determined by the turnover of NE stored in sympathetic nerves [12]. NE turnover reflects net leakage of NE from vesicular stores into the axoplasm and conversion of NE to DHPG. As a glycol, DHPG readily traverses cell membranes to enter the extracellular fluid and plasma. Plasma DHPG therefore provides a better indicator of NE turnover and the overall complement of noradrenergic nerves than does NE itself. DOPA is the immediate product of the enzymatic rate-limiting step in catecholamine biosynthesis, hydroxylation of tyrosine, and plasma DOPA is determined partly by tyrosine hydroxylase activity in sympathetic nerves [17]. Since the stored pool of NE is maintained by a balance of turnover and synthesis, plasma DHPG and DOPA levels normally are correlated with each other [18]. The finding of an elevated DOPA:DHPG ratio suggests increased catecholamine synthesis for a given amount of NE stores in sympathetic nerves.

In agreement with our previous report [11], FD patients had a distinct, abnormal pattern of plasma levels of catechols. When supine, the FD group had a normal mean plasma NE level but significantly decreased mean DHPG and increased mean DA and DOPA levels. The FD group also had high mean DOPA:DHPG, NE:DHPG, DA:DO-PAC, and DA:NE ratios. Taken together, these findings suggest an imbalance of decreased vesicular stores with increased catecholamine biosynthesis in sympathetic nerves and increased exocytotic release or decreased reuptake of catecholamine released from the decreased stores.

Because NE is converted to DHPG and DA to DOPAC via the actions of monoamine oxidase, one might ask whether high NE:DHPG and DA:DOPAC ratios in FD reflect

decreased monoamine oxidase activity. Conversion of DA to NE is catalyzed by DA-beta-hydroxylase, not monoamine oxidase, and both DHPG and DOPAC are deaminated metabolites. Decreased monoamine oxidase activity alone therefore would not explain high mean values for DA, DA:NE, or DOPAC:DHPG in FD.

We have now demonstrated that the previously observed abnormal catechol pattern in FD is relatively stable. Neither plasma levels of catecholamines nor of the other endogenous catechols changed significantly over a mean interval of 13 years in FD patients. The DOPA:DHPG ratio increased in all patients, by a mean of 24%. These findings lead us to infer that FD may involve a subtle further loss of noradrenergic nerves during adult life, with the effects mitigated partly by increased catecholamine biosynthesis in the remaining nerves.

Plasma levels of EPI tended to increase during long-term follow-up, but there was substantial inter-individual variability. Such a tendency might suggest a variable amount of compensatorily increased adrenomedullary secretion in response to decreased sympathetic noradrenergic innervation. Consistent with this explanation, chemical sympathectomy produced by 6-hydroxydopamine is associated with elevated plasma EPI concentrations [19].

With recent descriptions of in vitro agents that can rescue the mRNA *IKBKAP* splicing defect and enhance levels of functional ELP-1 in FD cell lines [20], pathophysiology-based treatment of this disease may evolve out of future clinical trials. The present findings suggest that plasma catechol profiling—in particular the DOPA:DHPG ratio—can provide biomarkers with which to track progression of the disease and effects of treatment.

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Abbreviations

DHPG	Dihydroxyphenylglycol
EPI	Epinephrine (adrenaline)
NE	Norepinephrine (noradrenaline)
DA	Dopamine
DOPA	3,4-dihydroxyphenylalanine
DOPAC	Dihydroxyphenylacetic acid
FD	Familial dysautonomia

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Fig. 1.

Individual values for the ratio of plasma dihydroxyphenylalanine (DOPA):dihydroxyphenylglycol (DHPG) as a function of plasma dopamine (DA):norepinephrine (NE) in control subjects (white circles), familial dysautonomia patients upon initial evaluation (gray circles), and familial dysautonomia patients after an interval of 13 years (black circles). Dashed lines placed to emphasize that all familial dysautonomia patients had both high DOPA:DHPG and high DA:NE ratios at both testing times, a finding not observed in any control subjects correlated positively with DA:NE ratios ($P = 0.69$, $P < 0.0001$).

Fig. 2. Individual values for the ratio of plasma dihydroxyphenylalanine (DOPA): dihydroxyphenylglycol (DHPG) in familial dysautonomia patients upon initial evaluation and after an interval of 13 years. Horizontal bars indicate mean values. Gray zone indicates values within 2 standard deviations of the control mean value. Note that all familial dysautonomia patients had an initial DOPA:DHPG ratio more than 2 standard deviations above the control mean and that the patients had a further increase in the DOPA:DHPG ratio at follow-up

Table 1

Plasma mean ($\pm$ SEM) levels of catechols (nmol/L) and catechol ratios in patients with familial dysautonomia (FD) and in control subjects. Data are shown for FD patients upon initial evaluation and after 13 years of follow-up

Compound	FD initial	FD follow-up	Control	<i>P</i> Initial, Follow-up
DA	0.14 $\pm$ 0.02	0.25 $\pm$ 0.06	0.03 $\pm$ 0.01	0.0004, 0.0002
NE	1.41 $\pm$ 0.17	1.76 $\pm$ 0.51	1.20 $\pm$ 0.10	0.21, 0.70
EPI	0.09 $\pm$ 0.03	0.49 $\pm$ 0.18	0.13 $\pm$ 0.02	0.06, 0.45
DOPA	9.68 $\pm$ 0.58	11.71 $\pm$ 1.71	7.93 $\pm$ 0.26	0.013, 0.0006
DOPAC	6.83 $\pm$ 2.47	7.56 $\pm$ 1.01	8.09 $\pm$ 0.70	0.07, 0.77
DHPG	2.53 $\pm$ 0.31	2.49 $\pm$ 0.43	4.92 $\pm$ 0.23	0.0004, 0.0004
DA:NE	0.10 $\pm$ 0.01	0.34 $\pm$ 0.23	0.02 $\pm$ 0.00	< 0.0001, 0.0003
NE:DHPG	0.59 $\pm$ 0.08	0.61 $\pm$ 0.12	0.25 $\pm$ 0.01	< 0.0001, < 0.0001
DA:DOPAC	0.029 $\pm$ 0.006	0.033 $\pm$ 0.009	0.004 $\pm$ 0.001	< 0.0001, < 0.0001
DOPA:DHPG	4.05 $\pm$ 0.32	4.89 $\pm$ 0.24 *	1.72 $\pm$ 0.06	< 0.0001, < 0.0001
DOPAC:DHPG	2.49 $\pm$ 0.57	3.35 $\pm$ 0.48	1.65 $\pm$ 0.12	0.03, < 0.0001

Notes: DA = dopamine; DHPG = dihydroxyphenylglycol; DOPA = L-3,4-dihydroxyphenylalanine; DOPAC = dihydroxyphenylacetic acid; EPI = epinephrine; NE = norepinephrine.

* Different from initial, $P = 0.015$