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Cardiac sympathetic hypo-innervation in familial dysautonomia

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Abstract

Objective—Familial dysautonomia (FD) involves incomplete development of the sympathetic nervous system. Whether such loss extends to sympathetic innervation of the heart has been unknown. This study used 6- ^{18}F fluorodopamine neuroimaging to assess cardiac sympathetic innervation and function in FD.

Methods—Six adult FD patients underwent thoracic PET scanning for 30 minutes after i.v. 6- ^{18}F fluorodopamine injection, as did healthy volunteers without ($N=21$) or with ($N=10$) pre-treatment by desipramine, which interferes with neuronal uptake and thereby simulates effects of noradrenergic denervation. Effective rate constants for uptake and loss were calculated using a single compartment pharmacokinetic model.

Results—FD patients had decreased uptake and accelerated loss of 6- ^{18}F fluorodopamine-derived radioactivity in the interventricular myocardial septum ($P=0.009$, $P=0.05$) and ventricular free wall ($P=0.007$, $P<0.001$), compared to untreated controls. Desipramine-treated subjects had decreased uptake but normal loss of 6- ^{18}F fluorodopamine-derived radioactivity.

Conclusions—FD involves cardiac noradrenergic hypo-innervation. Since accelerated loss of 6- ^{18}F fluorodopamine-derived radioactivity cannot be explained by decreased neuronal uptake alone, FD may also involve augmented NE loss from extant terminals.

Keywords

familial dysautonomia; fluorodopamine; sympathetic nervous system; positron emission tomography; norepinephrine

Introduction

Familial dysautonomia (FD), also known as Riley-Day syndrome and hereditary sensory and autonomic neuropathy type III (HSAN III), is a rare inherited disorder transmitted as an autosomal recessive trait in individuals of Ashkenazic extraction [1]. The etiologic basis of the disease is mutation of the gene, *IKBKAP*, with the most common mutation causing a splicing alteration that leads to tissue-specific decreased expression of IkappaB kinase-associated protein (IKAP), especially in neuronal tissue [19]. IKAP, 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 [3]. Many of the down-regulated genes are involved in cell migration, which may explain FD neuropathology that is consistent with arrested development of small nerve fibers.

Patients with FD usually have prominent sensory and autonomic abnormalities, such as impaired pain and temperature appreciation, orthostatic hypotension without compensatory tachycardia, episodic hyperhidrosis, gastroesophageal reflux, and decreased tear production. Central neural abnormalities include emotional lability and susceptibility to crises of vomiting, tachycardia, and paroxysmal hypertension. Other findings include oral incoordination, kyphoscoliosis, no axon flare reaction after intracutaneous histamine injection, and absence of fungiform lingual papillae. With supportive treatment, prognosis has improved greatly, so that about 40% of surviving patients are over age 20 years [2].

Adult patients with FD typically have orthostatic hypotension, associated with deficient orthostatic increments in plasma levels of the sympathetic neurotransmitter, norepinephrine (NE) [24]. Studies in the 1960s and 1970s noted relatively increased production of dopamine and its metabolites compared to NE and its metabolites [12, 21], consistent with decreased activity of dopamine-beta-hydroxylase, the enzyme catalyzing conversion of dopamine to NE. Plasma levels of dopamine-beta-hydroxylase activity initially were found to be decreased [23], but a subsequent report failed to confirm this finding [4].

Outside the brain, dopamine-beta-hydroxylase is localized to vesicles in sympathetic neurons and chromaffin cells. Post-mortem or biopsy studies have indicated that, rather than autonomic failure in FD reflecting dopamine-beta-hydroxylase deficiency, FD entails decreased numbers of sympathetic neurons. Blood vessels in renal and sural nerve biopsy tissue from FD patients have reduced sympathetic terminals [15, 16], and sympathetic ganglia contain decreased numbers of neurons [17].

The heart normally possesses abundant sympathetic innervation; however, neuropathological investigations have not been performed of this critical network in FD patients, a patient population known to have an increased incidence of sudden death [2]. 6-[¹⁸F]Fluorodopamine positron emission tomographic (PET) scanning can visualize cardiac sympathetic innervation [7] in vivo and may provide insights about the functional status of sympathetic nerves [10, 11].

In this study we carried out 6-[¹⁸F]fluorodopamine PET scanning in adult patients with FD and compared the results to those in healthy age-matched controls. Some control subjects were pretreated with desipramine, a tricyclic antidepressant that inhibits catecholamine

uptake by sympathetic nerves via the cell membrane NE transporter and thereby simulates decreased transporter activity due to denervation. We hypothesized that FD patients would have decreased density of sympathetic nerves in the myocardium that could be demonstrated by decreased uptake of 6-[¹⁸F]fluorodopamine.

Methods

Six adult patients with FD (mean age 33 ± 4 (SEM) years, 2 males 4 females) underwent thoracic positron emission tomographic (PET) scanning for 30 minutes after i.v. injection of 6-[¹⁸F]fluorodopamine at the NIH Clinical Center. All the patients had been diagnosed at and were followed by the Familial Dysautonomia Clinic of the New York University Medical Center. All the patients were tested while on their usual medications. None was taking any medication known to affect the neuronal uptake of catecholamines.

Thoracic PET scanning was conducted as described previously [7]. Briefly, 6-[¹⁸F]fluorodopamine (1 mCi) was injected i.v. over 3 minutes using an automated syringe pump. Dynamic 3-D data were acquired for 30 minutes from the time of initiation of the injection. Regions of interest were placed in the interventricular septum, apex, and free wall, and time-activity curves were generated from the average radioactivity in each region of interest. Radioactivity (in nCi/cc) was adjusted for the 6-[¹⁸F]fluorodopamine dose per unit body mass (mCi/kg) and therefore expressed in units of nCi-kg/cc-mCi. There were 9 scanning frames, with the midpoints at about 0.5, 1.5, 2.5, 3.5, 8, 13, 18, and 26 minutes from the start of injection.

In each subject, a mono-exponential curve of best fit was generated for the concentrations of 6-[¹⁸F]fluorodopamine-derived radioactivity in the septum and left ventricular free wall, at the time of peak radioactivity (usually in the 8 minute frame). The y -intercept (y_0) value was taken as an index of the uptake of 6-[¹⁸F]fluorodopamine by sympathetic nerves, and the rate constant for loss of radioactivity (k_{loss}) was taken as an index of rapid exchange between the vesicles and extracellular fluid [11].

The neuroimaging data in the FD group were compared with those in a previously studied group of untreated healthy volunteers ($N=21$, mean age 31 ± 1 years, 16 males 5 females) and in a previously studied group of healthy volunteers ($N=10$, mean age 42 ± 3 years, 9 males 1 female) who were pre-treated with oral desipramine about 1.5–2 hours before 6-[¹⁸F]fluorodopamine injection. Desipramine inhibits uptake of catecholamines, including 6-[¹⁸F]fluorodopamine, by sympathetic nerves.

Mean values were compared between the FD and control group and between the desipramine-treated and untreated groups by independent-means t tests. All mean values were expressed $\pm$ SEM.

Results

FD patients had diffusely decreased 6-[¹⁸F]fluorodopamine-derived radioactivity throughout the left ventricular myocardium or had heterogeneously decreased radioactivity, with greater loss in the apex or inferolateral free wall than in the anterobasal septum (Figure 1).

Time-activity curves for myocardial 6-[¹⁸F]fluorodopamine-derived radioactivity indicated decreased uptake in the FD group, compared to the untreated control group, and accelerated loss compared to both treated and untreated control groups (Figure 2). When compared to untreated controls, FD patients had decreased uptake of 6-[¹⁸F]fluorodopamine, as reflected by a low mean value for y_0 , in both the septum ($7,952 \pm 1,054$ vs. $10,792 \pm 423$ nCi-kg/ccmCi in untreated controls, $P = 0.009$) and free wall ($6,985 \pm 900$ vs. $10,151 \pm 487$ nCi-kg/cc-mCi in untreated controls, $P = 0.007$); however, the decrease in uptake was not as marked as in the desipraminetreated subjects as the curve relating myocardial 6-[¹⁸F]fluorodopamine-derived radioactivity with time (time-activity curve) was displaced downward compared to that in FD patients and the untreated control subjects (Figure 2). In neither the FD patient group nor the untreated control group did the mean value for y_0 differ between the septum and free wall. Desipramine-treated subjects, had a low mean value for y_0 in the septum ($5,850 \pm 586$ nCi-kg/cc-mCi, $P < 0.001$ compared to untreated subjects).

Time-activity curves also indicated that the FD group had accelerated loss of 6-[¹⁸F]fluorodopamine-derived radioactivity, compared to both the treated and untreated control groups, resulting in a visibly steeper slope than in the control groups (Figure 2). FD patients had accelerated loss of 6-[¹⁸F]fluorodopamine-derived radioactivity, as indicated by a high mean value for k_{loss} , in both the septum (0.026 ± 0.002 vs. 0.020 ± 0.001 min⁻¹ in untreated controls, $P = 0.05$) and free wall (0.029 ± 0.002 vs. 0.018 ± 0.001 min⁻¹ in untreated controls, $P < 0.001$). Desipramine-treated subjects had about the same mean value for k_{loss} (0.022 ± 0.003 min⁻¹) as in untreated control subjects.

Discussion

The present neuroimaging findings provide in vivo neuroimaging evidence that FD involves decreased cardiac sympathetic innervation. Previous studies described diminished sympathetic neuronal populations in biopsies of renal blood vessels and sural nerve [15, 16], and decreased numbers of neurons in sympathetic ganglia harvested post-mortem.

Consistent with decreased numbers of cardiac sympathetic neurons, at all time points after injection of the sympathetic neuroimaging agent (6-[¹⁸F]fluorodopamine) myocardial concentrations of 6-[¹⁸F]fluorodopamine-derived radioactivity were decreased by about 25% in the interventricular septum and about 30% in the left ventricular free wall, compared to values in untreated control subjects. These percent decreases were smaller than those found in healthy volunteers pre-treated with desipramine (about 45% in the interventricular septum). FD, therefore, may entail a quantitative decrease in the density of cardiac sympathetic nerves, rather than a complete absence of innervation; although two of the six patients did have markedly decreased 6-[¹⁸F]fluorodopamine-derived radioactivity throughout the left ventricular myocardium.

Accelerated loss of myocardial 6-[¹⁸F]fluorodopamine-derived radioactivity, compared to both untreated and desipramine-treated control subjects, indicates a functional abnormality in the extant sympathetic nerves. The augmented loss of radioactivity is not explained by decreased neuronal reuptake alone, because desipramine treatment did not accelerate the loss of radioactivity in healthy volunteers. Within a few minutes after i.v. injection of 6-

[¹⁸F]fluorodopamine, virtually all the 6-[¹⁸F]fluorodopamine-derived radioactivity in the myocardium reflects uptake by sympathetic nerves, since destruction of those nerves, by 6-hydroxydopamine injection, results in inability to distinguish radioactivity in the myocardium from that in the left ventricular chamber [8]. Moreover, intra-neuronal 6-[¹⁸F]fluorodopamine is taken up rapidly by and concentrated in vesicles in sympathetic nerves, via the vesicular monoamine transporter, because reserpine treatment also results in markedly decreased myocardial radioactivity [8]. The increased rate of loss of myocardial 6-[¹⁸F]fluorodopamine-derived radioactivity in FD therefore seems to reflect a greater net rate of exchange of the vesicular contents with the extracellular fluid. The proportion of vesicles in a high-probability release state might be increased, such as via compensatorily increased traffic in extant nerves, with more exocytosis of the vesicular contents into the extracellular fluid. Alternatively, since inhibition of the vesicular monoamine transporter accelerates the loss of 6-[¹⁸F]fluorodopamine-derived radioactivity [8], the present results do not exclude the possibility of decreased vesicular monoamine transporter efficiency in FD, with loss of 6-[¹⁸F]fluorodopamine-derived radioactivity via oxidative deamination to 6-[¹⁸F]fluorodihydroxyphenylacetic acid in the axoplasm.

Either of these in vivo functional changes would fit with previous post-mortem reports demonstrating increased tyrosine hydroxylase immunoreactivity in sympathetic ganglia of patients with FD [14], despite decreased numbers of ganglionic neurons [17]. Although decreased urinary excretion of radiolabeled NE and its metabolites after administration of ¹⁴C-dopamine was taken as indicating decreased NE synthesis [12], this finding could be explained by sympathetic hypo-innervation or by decreased vesicular uptake of dopamine. In Parkinson disease, which also entails a decrease in cardiac sympathetic innervation [9], there is increased washout of ¹²³I-metaiodobenzylguanidine-derived radioactivity [13, 22], consistent with a functional change analogous to that noted here in FD patients. ¹²³I-Metaiodobenzylguanidine is not a substrate for monoamine oxidase. Assuming that the same functional change occurs in FD as in PD, the available evidence would seem to favor compensatorily increased exocytosis over decreased vesicular sequestration as the mechanism of accelerated loss of 6-[¹⁸F]fluorodopamine-derived radioactivity in FD.

Patients with FD seem to have an increased risk of tachy- and brady-arrhythmias, asystole, and sudden death [5, 6, 18]. Our finding of cardiac hypoinnervation provides a possible explanation for arrhythmias in FD. A developmental abnormality of cardiac sympathetic innervation, involving areas with approximately normal innervation nearby areas without innervation, could set the stage for re-entry type tachy-arrhythmias from heterogeneous conduction or heart block or asystole from unopposed vagal tone.

FD is thought to be a progressive disorder. Whether preadolescent patients would have the same degree of hypo-innervation as adults remains unknown; however, since there was essentially no difference between the findings in a 21- and a 50-year-old patient in our study, the hypo-innervation may be mainly a developmental problem.

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 in vivo cardiac neuroimaging, along with neurochemical profiles, may be biomarkers with which to track effects of treatment.

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Fig. 1.

(*Top*) Thoracic transaxial PET images after 6- ^{18}F fluorodopamine i.v. injection into a healthy control subject (*Normal*), a patient with diffuse sympathetic denervation due to pure autonomic failure (*PAF*), and a patient with familial dysautonomia (*FD*). Note decreased 6- ^{18}F fluorodopamine-derived radioactivity in the left ventricular myocardium of the *FD* patient. (*Bottom*) Left lateral (*L Lat.*), left anterior oblique (*LAO*), anterior (*Ant.*), right anterior oblique (*RAO*), and right lateral (*R Lat.*) views of the heart in an *FD* patient. *Dashed lines* indicate approximate perimeter of the heart. *Arrows* indicate regions of decreased 6- ^{18}F fluorodopamine-derived radioactivity in the inferolateral wall and apex

Fig. 2.

Mean ($\pm$ SEM) values for (*left*) interventricular septal and (*right*) left ventricular free wall myocardial 6- 18 F]fluorodopamine-derived radioactivity, in normal control subjects (*black circles*), patients with familial dysautonomia (FD, *white circles*), and desipramine (DMI)-pretreated control subjects (*gray circles*). Note decreased radioactivity in FD at all time points, compared to untreated control subjects, with more rapid decline in radioactivity in the FD group compared to both untreated and desipramine-treated control subjects.