Heart norepinephrine content in iron deficiency anemia

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*Rat heart norepinephrine (NE) content is significantly diminished and NE fractional turnover is decreased by dietary iron deficiency anemia at the same time that left ventricle size nearly doubles (*Proc. Soc. Exp. Biol. Med. *193:306–312, 1990). To determine if this low NE content is due to a) impaired synthesis per se, or b) mismatching of synthesis relative to adrenergic impulse traffic; we performed several experiments in which nerve traffic was altered through the use of a ganglionic blocking drug. In addition, we examined tyrosine hydroxylase activity both in vivo and in vitro. Heart NE concentration was 50% less than normal in the hypertrophied iron-deficient hearts (146 ± 59 versus 481 ± 72 ng NE/g). Ganglionic blockade for three hours resulted in similar elevations in NE contents in both dietary treatments. Pharmacologic inhibition of aromatic amino acid decarboxylase resulted in similar rates of dihydroxy phenyl alanine (DOPA) accumulation in hearts of anemic and control rats demonstrating equivalent in vivo tyrosine hydroxylase activities. In addition, in vitro tyrosine hydroxylase activity was similar. These findings demonstrate that norepinephrine synthesis per se is not defective in the iron-deficient heart but may still be limited in its capacity to match the demands of higher sympathetic activity.*

Keywords: iron deficiency; heart hypertrophy; anemia; norepinephrine synthesis

Introduction

Norepinephrine (NE) metabolism in peripheral organs is significantly affected by nutritional iron deficiency.[1-3] NE mass turnover rate and NE content are significantly lower in hearts of iron-deficient rats compared with iron-sufficient controls while the fraction of the NE pool turning over per unit of time is significantly increased.[1,2] The severity of the depletion of NE is correlated with the severity of anemia and the increase in left ventricle size. Plasma NE concentrations are elevated in iron-deficient humans[4-6] and animals[2,7,8] to a similar degree as cardiomyopathy patients.[9,10] Elevated efferent sympathetic nervous system (SNS) activity and/or altered uptake and catabolic processing of the neurotransmitter have been noted as characteristics of these failing hearts. Iron deficiency is not unique in causing changes in heart NE content. Copper is an essential cofactor for dopamine-β-hydroxylase, and with copper deficiency, changes in heart NE turnover and content are similar to what we have observed in iron deficiency.[11]

The purpose of the present study was to estimate NE synthetic capacity both in vivo and in vitro after a severe condition of iron deficiency has been established. In addition, we wanted to examine the possibility that heart NE content in iron deficiency was diminished due to an extremely high sympathetic drive and a relative limited synthetic capacity. Because tyrosine-3-hydroxylase activity is a non-heme iron dependent enzyme,[12] dietary iron deficiency that is quite severe could limit the synthesis of this neurotransmitter. This limitation may be relative because high rates of SNS activity in the anemic state could exceed synthesis and recycling capacity with a subsequent decrease in content. This seems reasonable, given the increased cardiac work demonstrated in severe anemia and the hypertrophy associated with that increased work.[3,13] Thus, the amount of NE synthesis obtained when the ganglionic chain is intact represents the ability of synthesis to maintain a constant NE pool size with neuronal activity. When neural firing is inhibited by ganglionic blockade, synthesis represents the relative ability of the heart to establish a new set point

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of NE content without the influence of nerve traffic on norepinephrine content.

Methods

Animals

Male, weanling Sprague-Dawley rats (Hilltop Labs, Denver, PA and Harlan Sprague-Dawley, Indianapolis, IN, USA) were obtained at 21 days of age and housed individually in stainless steel cages with a 12-hour light:dark cycle (lights on at 0700 hr). Animals were assigned to either iron-deficient or control dietary treatments. All animals received a modified AIN-76 diet formulation.[14,15] The control diet had 50 ppm iron added as ferrous sulfate, while the iron-deficient diet had no added iron (<2 ppm Fe). Corn starch rather than sucrose was the dietary carbohydrate source as recommended by the American Institute of Nutrition[15] for studies in which sucrose may affect the dependent variable. Similarly, due to variable iron content, non-nutritive cellulose was deleted from the diets of both groups.

Feed and distilled deionized water were available ad libitum. All procedures were approved by The Pennsylvania State University Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines.

Pharmacologic interventions

Animals were maintained on respective diets for 5 weeks during which time experiments were carried out to assess tissue NE content in the heart following: (1) ganglionic firing inhibition, (2) NE synthesis inhibition, (3) both ganglionic inhibition and NE synthesis inhibition, and (4) saline injection. All animals were sacrificed on the same day, with all treatments evenly spaced throughout the day to minimize any diurnal effect on tissue NE content.

Ganglionic inhibition was obtained using an intraperitoneal injection of chlorisondamine (C, 5 mg/kg body weight, dissolved in sterile 0.9% saline) 165 minutes and 90 minutes prior to sacrifice. Dose and timeframe were established in a pre-test such that a significant lowering in metabolic rate and establishment of a plateau occurred within 30 minutes of this dose and persisted for over 2 hours. The minimal drug concentration needed to induce this alteration by ganglionic blockade was then used in the described studies. Treatment groups not receiving chlorisondamine received injections of sterile saline 165 minutes and 90 minutes prior to sacrifice. Inhibition of NE synthesis was obtained via intraperitoneal injection of the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine methyl ester (AMPT, 250 mg/kg body weight, dissolved in 0.9% sterile saline) given 180 minutes prior to sacrifice. Animals not receiving AMPT received an intraperitoneal saline injection 180 minutes prior to sacrifice. AMPT is useful for this type of study in that its effects are rapid, complete, and do not affect other aspects of tissue NE metabolism.[16,17]

The resultant treatment groups were:

ID-C-S	Iron deficient, chlorisondamine, no AMPT (saline)
ID-C-AMPT	Iron deficient, chlorisondamine, AMPT
ID-S-S	Iron deficient, no chlorisondamine (saline), no AMPT
ID-S-AMPT	Iron deficient, no chlorisondamine, AMPT
CN-C-S	Control, chlorisondamine, no AMPT
CN-C-AMPT	Control, chlorisondamine, AMPT
CN-S-S	Control, no chlorisondamine, no AMPT
CN-S-AMPT	Control, no chlorisondamine, AMPT

In a separate experiment, iron-deficient and control rats were injected intraperitoneally with saline or an aromatic amino acid decarboxylase inhibitor, m-hydroxy-benzylhydrazine, 45 minutes before sacrifice.[11] The drug was prepared in sterile isotonic saline, ph 7, and injected intraperitoneally (100 mg/kg body weight). The accumulation of DOPA within this time frame is linear and is an estimate of in vivo tryrosine hydroxylase activity.

Tissue collection

Animals were sacrificed by decapitation. Trunk blood was collected into heparinized tubes, and hemoglobin and hematocrit were determined using cyanomethemoglobin and microcapillary methods, respectively. Hearts were quickly removed, frozen immediately in liquid nitrogen, and stored at −80°C for later analysis (no more than 3 weeks).

Tyrosine hydroxylase activity

In some rats from each dietary treatment group, the hearts were used for the measurement of in vitro tyrosine hydroxylase according to published procedures without the addition of exogenous iron to the incubation medium.[10] These animals were not injected with either drugs or saline and assays performed within the 4–6 hours of decapitation.

Catecholamine determination

Tissues were prepared for extraction and determination of catecholamines using previously published and well described methods employing electrochemical detection and HPLC reverse phase chromatography.[1,2] Dihydroxybenzylamine was used as the internal standard for both DOPA and NE extraction efficiency calculations. This efficiency averaged 78% ± 4%. To assess the relative contributions of synthetic capacity and ganglionic firing innervation to the tissue NE pool, several published intermediary calculations were made.[18]

Statistical analysis

Data were analyzed by three-way analysis of variance using the general linear systems model of SAS (SAS Version 5.1, Statistical Analysis Systems, Cary, NC, USA). Class variables were iron status, chlorisondamine treatment, and AMPT treatment with all main effect interaction permutations calculated. Type III sums of squares were used in the determination of significance for main and interactive effects. A post hoc least squares means test was used to assign differences between groups. Significance was assigned to a $P \leq 0.05$ level. All data (unless otherwise noted) are presented as arithmetic mean ± the standard deviation.

Chemicals and reagents

Vitamin test casein and AIN-76 vitamin mix were obtained from ICN Nutritional Biochemicals (Cleveland, OH, USA). Corn oil and corn starch were obtained from a local supplier. Chlorisondamine was the generous gift of the CIBA Geigy Corporation, Pharmaceutical Division (Suffern, NY, USA). AMPT and m-hydroxy-benzyl hydrazine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dihydroxybenzylamine and acid washed alumina were obtained from Bioanalytical Systems, Inc., West Lafayette, IN, USA. All other chemicals were reagent grade and obtained from the Sigma Chemical Company.

Results

A low-iron diet resulted in a significantly lower body weight and a severe anemia after 5 weeks when compared with the control dietary treatment (*Table 1*). Iron-deficiency anemia was also associated with significantly increased heart weight:body weight ratio (*Table 2*). NE content and concentrations were 61%–79% lower in all iron-deficient treatment groups when compared with respective controls. Chlorisondamine treatment resulted in significantly higher heart NE content in iron-deficient and control groups 3 hours after administration of the ganglionic blocking drug.

Calculations of synthetic and ganglionic contributions to heart NE content and concentration are shown in *Table 3*. In terms of absolute increase in NE concentration, when sympathetic firing was blocked for 3 hours, iron deficient and control animals had similar readjustments in pool size, 110 and 134 ng NE, respectively (*Table 3*). When synthesis is inhibited at the same time that the ganglionic blocker presumably causes a diminution in nerve traffic, pool size increased only 73 and 87 ng NE. This suggests an avid and nearly equal accumulation of NE from the extracellular pool in both iron-deficient and control rats. The synthetic contribution to the NE pool is calculated in a fashion analogous to calculations of NE turnover following α MPT injection. These calculations showed iron deficient animals to have 24% slower rates of synthesis (65 ng per 3 hours compared with 86 ng per 3 hours in controls) when the ganglionic blocker drug is present and equivalent rates when the blocker is absent.

In vitro measurements of tyrosine hydroxylase activity provides a second means of estimating synthetic capacity (*Table 4*). There was no statistical difference between iron-deficient and control animals when this rate limiting step in NE synthesis was examined in a large cohort of animals.

A third route of examination of heart NE synthesis was examined by inhibiting the heart aromatic amino acid carboxylase activity and determining the rate of in vivo accumulation of L-DOPA (*Table 5*). Severely anemic rats synthesize L-DOPA as rapidly as control animals (2.15 ± 0.12 $ng \cdot min^{-1}$ versus 1.70 ± 0.18 $ng \cdot min^{-1}$ in controls) despite a 50% lower heart NE content than control rats (468 ± 64 ng NE versus 1009 ± 168 ng NE).

Table 1 Effect of iron deficiency on body weight and hematologic parameters

	Body weight (g)	Hematocrit (% PCV)	Hemoglobin ($g \cdot dL^{-1}$)
CN (n = 20)	223 ± 13	45 ± 2	11.4 ± 1.6
ID (n = 20)	174 ± 14	16 ± 2	3.4 ± 0.5

Discussion

This study demonstrates that severely anemic iron-deficient rats have a dramatic lowering in heart NE content during heart hypertrophy. This heart NE pool rapidly returns toward normal with chlorisondamine treatment and a presumed diminution of nerve traffic. In vitro assessments of tyrosine hydroxylase activity agree with the in vivo estimate of activity by showing little deleterious effect of iron deficiency on enzyme activity. In addition, when aromatic amino acid decarboxylase is inhibited in vivo, L-DOPA accumulates at nearly equal rates in anemic and control rats, further demonstrating that tyrosine hydroxylase in vivo is not rate limiting in iron deficiency within the constraints of these experiments.

Table 2 Effect of iron deficiency, chlorisondamine (ganglionic firing inhibition) treatment, and AMPT (norepinephrine synthesis inhibition) on heart weight and norepinephrine content

	Heart Weight (g)	Heart:body weight ratio ($g \cdot kg^{-1}$)	Norepinephrine Concentration ng NE $\cdot g^{-1}$	Norepinephrine Content ng NE $\cdot$ tissue^{-1}
CN-C-S	0.85 ± 0.02^{a}	3.81 ± 0.26^{c}	723 ± 177^{c}	615 ± 142^{c}
ID-C-S	0.90 ± 0.06^{abc}	5.32 ± 0.60^{a}	282 ± 54^{a}	256 ± 57^{a}
CN-C-AMPT	0.84 ± 0.02^{ab}	3.67 ± 0.24^{c}	632 ± 149^{cd}	529 ± 118^{cd}
ID-C-AMPT	0.95 ± 0.09^{ace}	5.43 ± 0.42^{ab}	204 ± 67^{ab}	191 ± 56^{ab}
CN-S-S	0.84 ± 0.06^{ab}	3.80 ± 0.14^{c}	575 ± 76^{d}	481 ± 72^{d}
ID-S-S	1.02 ± 0.17^{cd}	5.89 ± 0.56^{b}	148 ± 67^{b}	146 ± 59^{b}
CN-S-AMPT	0.82 ± 0.10^{b}	3.79 ± 0.22^{c}	538 ± 73^{d}	442 ± 98^{d}
ID-S-AMPT	1.04 ± 0.15^{de}	5.88 ± 0.47^{b}	115 ± 54^{b}	118 ± 55^{b}
AOV				
Iron status	0.0001*	0.0001	0.0001	0.0001
Chlorisondamine	NS	0.0235	0.0005	0.0005
AMPT	NS	NS	NS	0.0464

Groups not sharing a superscript letter in a column are significantly different by least-squares means ($P < 0.05$) comparisons.
Data presented as mean ± S.D.
* The interaction term of iron status and chlorisondamine treatment was significant with $P = 0.0351$.

Table 3 Estimated synthetic and ganglionic contributions to the heart norepinephrine pool size of iron deficient and control animals*

	ng·g^{-1}	ng·tissue^{-1}
Synthetic contribution		
Chlorisondamine present		
CN	91	86
ID	78	65
Chlorisondamine absent		
CN	37	39
ID	33	28
Ganglionic contribution		
Synthesis Inhibited		
CN	94	87
ID	89	73
Synthesis Intact		
CN	148	134
ID	134	110

* Synthetic capacity is the difference in norepinephrine content when synthesis is intact and when it is inhibited. Ganglionic contribution is the difference in norepinephrine content when ganglionic firing is intact and the content when firing is inhibited.[18]

Table 4 Effect of iron deficiency on in vitro tyrosine hydroxylase activity*

	Tyrosine hydroxylase activity† (pmoles DOPA·g tissue^{-1}·hr^{-1})
CN	2.01 ± 0.70 (n=19)
ID	2.53 ± 0.61 (n=17)

* These iron deficient and control animals are a separate cohort but with identical dietary treatments as other animals.
† Mean ± SEM

Table 5 Effect of inhibition of aromatic amino acid carboxylase on L-DOPA content in hearts of iron deficient and control rats*

	0′	45′ after inhibition
	L-DOPA heart contents (ng/heart)	
ID	25 ± 4	122 ± 42
CN	30 ± 6	107 ± 16

* Animals were fed their respective diets for 8 weeks. Iron deficient animals had a mean body weight of 231 ± 21 and controls = 297 ± 20 g. Hemoglobin concentration averaged 7.2 ± 0.9 and 15.3 ± 5 g/dL, respectively. Ventricles averaged 433 ± 33 mg per 100 gm body weight in iron deficient animals and only 349 ± 34 in controls. NE content was significantly lower (468 ± 64 ng) in iron deficient than control animals (1009 ± 168).

The postganglionic sympathetic neuronal norepinephrine pool size is normally maintained by matching rates of synthesis and reuptake to neurotransmitter release due to neural depolarization.[19] The depletion of norepinephrine within the heart in iron deficiency could thus be due to one or more of the following: tyrosine delivery and uptake into the tissue;[20] poor coupling of catecholamine synthesis to pool size; inappropriate neuronal release of NE due to an effect of presynaptic α adrenoceptors;[21] and decreased reuptake and reutilization of NE.[22-24]

The first possibility is unlikely given the substantial protein component of the diet and tyrosine content of casein and the identical nature of the diets except for iron content.[14] The second and third possibilities are examined in this study. The fourth and fifth are relatively unexamined in iron deficiency and are the basis of ongoing and future experiments.

In returning to the issues of synthesis and release of NE in iron-deficient hearts, iron deficiency in the rat clearly results in a severe cardiac hypertrophy and decreased heart NE content.[1,3,11] While the pathogenesis of the hypertrophy is still not clear, it seems probable that the myocardial enlargement is a physiologic attempt to maintain oxygen delivery to peripheral tissues in anemic animals. Heart NE content is known to fall dramatically in the failing heart[25] with a possible explanation being a change in extraction of NE from the synaptic cleft back into the neuron.[23] This leads to an increased spill-over into the plasma and elevations in plasma NE.[24] Interestingly, iron deficient rats are known to have elevations in plasma and urine NE.[7,8]

While it is not correct to calculate NE turnover from data generated in these experiments, we have shown in other studies that the loss of NE in the heart after AMPT administration does follow the expected monoexponential decline.[1,2,26] If we assume the current animals are not really dissimilar to those in previous studies, we can calculate a fractional rate of loss of NE averaged 8.4% in iron-deficient animals and 2.2% in controls; a difference similar to our other studies.[1,2] Because AMPT inhibits NE synthesis prior to incorporation into vesicles, this estimation of turnover would not accurately evaluate NE cycling from vesicles to axoplasm with some irreversible conversion to dihydroxyphenylglycol. Nor does it allow an estimate of the efficiency of recapture of NE from the synaptic cleft.[27]

Regardless of sympathetic activity in the heart (eg, with or without chlorisodamine) iron-deficient animals are losing proportionally greater amounts of NE from tissue than controls and in agreement with increased fractional turnover rates.[1,2,27] Given the similarity of these observations to those made in examinations of NE metabolism in heart failure;[24] we suggest that an alteration in recycling in combination with some heretofore unexplained limitation in synthesis keeps norepinephrine content very low in severe anemia. The limitation of not knowing the actual efferent neural firing rates or the density of sympathetic neurons may be serious limitations to our logic, though the previously established morphology of the hypertrophied iron deficient rat heart[3] suggests a heart in heart failure.[28] Extraordinarily high neuron firing rates and a decreased density of nerves may still be explanations; nonetheless, three different types of experiments all suggest that synthesis is not impaired.

In summary, these experiments demonstrate that a

brief pharmacologic in vivo interruption in neuronal firing rate causes a dramatic and equivalent increase in NE content in iron deficient anemic and control rat hearts. When considered with in vitro and in vivo assessments of tyrosine hydroxylase activity, we conclude that NE synthetic capacity per se is not limiting the NE content of the hypertrophied iron-deficient heart.

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