Kynurenic acid, an aryl hydrocarbon receptor ligand, is elevated in serum of Zucker fatty rats

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Abstract

Obesity is an increasingly urgent global problem. Molecular mechanisms of obesity have not been fully understood. Dysregulation of tryptophan (Trp) – kynurenine (Kyn) metabolic pathway (TKP) was suggested as one of the mechanism of obesity and described in obese subjects and animal models of obesity. However, to the best of our knowledge, TKP metabolism has not been studied in leptin-receptor-deficient Zucker fatty rats (ZFR) (fa/fa), the best-known and most widely used rat model of obesity. We therefore interested to find out if there are any deviations of TKP in ZFR. Concentrations of major TKP metabolites were evaluated (HPLC- MS method) in serum of ZFR (fa/fa) and lean rats (FA/-). Concentrations of kynurenic acid (KYNA) were 50% higher in ZFR that in lean rats (p<0.004, Mann-Whitney two-tailed test). While elevation of anthranilic acid (AA) concentrations (33%), did not reach high level of statistical significance (p<0.04, one-tailed test). Our data suggested that elevated KYNA serum concentrations might contribute to development of obesity via KYNA-induced activation of aryl hydrocarbon receptor (AHR) and to cognitive impairment in ZFR because of KYNA antagonism to N-methyl-d-aspartate receptor (NMDAR). Elevated KYNA concentrations were reported in brains, cerebrospinal fluid and serum of schizophrenia patients. Therefore, up-regulated KYNA formation might contribute to high prevalence of obesity in schizophrenia patients. Present results warrant further studies of KYNA and AA in other animal models of obesity.

Introduction

Obesity is an increasingly urgent global problem. Molecular mechanisms of obesity have not been fully understood. Dysregulation of tryptophan (Trp) – kynurenine (Kyn) metabolic pathway (TKP) was suggested as one of the mechanisms to of obesity [1,2]. In mammals, TKP consists of three major phases: initial conversion of Trp into KYN (via N-formylKYN) catalyzed by inflammation-induced indoleamine-2,3-dioxygenase 1 (*IDO*) or stress-activated Trp-2,3-dioxygenase 2 (*TDO*); intermediate by Kyn conversion into 3-hydroxykynurenine (3-HK), kynurenic (KYNA) and anthranilic (AA) acids; and final phase production of NAD initiated by 3-HK conversion into 3-hydroxyAA (Figure 1) [3]. KYNA and AA are the end-products of KTP in astrocytes and adipocytes because Kyn-3-monooxygenase (*KMO*), a riboflavin (vitamin B2)-dependent enzyme, that catalyzes Kyn conversion into 3-HK, is not expressed in these tissues [4,5].

Activation of TKP initial phase was reported in animal models of obesity [6,7] and in obese human subjects [8-10]. Recent studies found different patterns of TKP dysregulation in obese mice and in human obesity and concluded that these mouse models [high-fat diet induced-obesity and the leptin-deficiency (ob/ob)] are inappropriate for studies of TKP involvement in mechanisms of human obesity [11]. However, to the best of our knowledge, TKP metabolism has not been studied in leptin-receptor-deficient Zucker fatty rats (ZFR) (*fa/fa*), the best-known and most widely used rat model of obesity. We were interested to find out if there are any deviations of TKP in ZFR by comparing serum concentrations of major TKP metabolites in ZFR (fa/fa) and lean rats (*FA/-*).

Methods

Serum samples (drawn after 5 hrs of fasting) from male ZFR (fa/fa) and lean (*FA/-*) rats (6 – 8 weeks of age) were provided by Charles River, Inc. and stored at −50°C until analysis. Trp, Kyn, KYNA, AA, 3-HK, and XA were analyzed by modified HPLC – MS method [12,13].

Statistical analysis

Results are presented as mean ± standard error (Trp and Kyn in µM and AA, KYNA, 3-HK and XA in nM). Statistical significance of differences between lean and ZFR (six rats in each group) was assessed by Mann-Whitney test.

Results

Initial phase of TKP. There were no difference of Trp and Kyn serum concentrations between ZFR and lean rats (Table 1).

Kyn:Trp ratio (an indirect marker of activity of enzymes catalyzing Trp conversion into Kyn) was not different between ZFR and lean rats (Table 1).

Intermediate phase of TKP. Serum concentrations of KYNA were elevated (by 50%) in ZFR (Table 1). There was a strong tendency to elevation of AA concentrations (by 33%). 3-HK concentrations did not differ between ZFR and lean rats.

Final phase of TKP. Concentrations of XA, a suggested diabetogenic 3-HK metabolite [17], did not differ between ZFR and lean rats.

Table 1. Kynurenines (serum) concentrations in Zucker fatty and lean rats.

N=6	Lean	Obese	P*	P**
Trp (μM)	133.60 ± 6.64	116.80 ± 4.14	ns	
Kyn (μM)	1.89 ± 0.14	1.87 ± 0.16	ns	
AA (nM)	60.08 ± 6.74	80.96 ± 15.55	0.08	0.04**
KYNA (nM)	70.06 ± 2.77	105.05 ± 9.98	0.004*	0.002**
3HK (nM)	19.23 ± 0.98	20.57 ± 1.84	ns	
XA (nM)	13.10 ± 1.50	14.21 ± 1.54	ns	
Kyn/Trp	1.41 ± 0.09	1.60 ± 1.01	ns	

P* - Mann-Whitney : P* - two-tailed test; P** - one-tailed test.
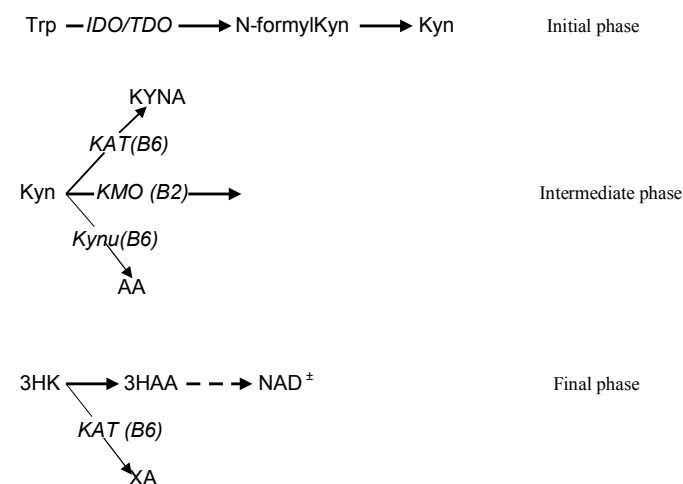
Figure 1. Three major phases of Tryptophan – Kynurenine metabolic pathway.

Abbreviations: Trp: tryptophan; Kyn: kynurenine; KYNA: kynurenic acid; 3HK: 3-hydroxykynurenine; AA: anthranilic acid; 3HAA: 3-hydroxyanthranilic acid; XA: xanthurenic acid (3-hydroxyKYNA); NAD^+: nicotinamid adenine dinucleotide; KMO: kynurenine 3-monooxygenase; Kynu: kynureninase; KAT: kynurenine aminotransferase 2

Discussion

Present finding of elevated concentrations of serum KYNA (and, probably, of AA) in ZFR is important because, to the best of our knowledge, it is the first recognition of KTP dysregulation in ZFR. Particularly we found, dysregulation of the intermediate phase of KTP in ZFR.

Our finding is in line with reported positive correlation of serum KYNA concentrations with BMI in obese subjects [4,14].

Elevation of serum KYNA concentrations might result from up-regulated KYNA biosynthesis in fat tissue, liver and minocytes/macrophages. Formation of KYNA and AA from Kyn is catalyzed by B6 (PLP)-dependent *KAT* and *Kynu*, respectively (Figure 1). However, since *KAT* and *Kynu* are substrate-unsaturated enzymes, they are able to process additional amount of Kyn created by *KMO* inhibition. Thus, KYNA and AA were elevated in brain, liver and plasma of *KMO*(-/-) mice [15]. Furthermore, increased formation of KYNA and AA without activation of *KAT* and *Kynu* was observed in baboons and mice fed by vitamin B2 (but not vitamin B6) deficient diets [16,17]. Therefore, KYNA elevation in serum of ZFR may be a consequence of *KMO* deficiency in fat tissue that do not express *KMO* genes [4]. On the other hand, KYNA might be synthesized by resident macrophages infiltrating omental adipose tissue women with obesity [4]. Therefore, *KMO* inhibition and/or *Kynu* activation may contribute to our observation of elevated serum KYNA (and AA) in ZFR. Identification of origin of serum KYNA elevation in ZFR needs further studies.

Functional implications of elevated KYNA in ZFR could depend on KYNA antagonism to N-methyl-d-aspartate (NMDAR) and alpha7 nicotinic acetylcholine receptors (α7nAChR) and activation of aryl hydrocarbon receptor (AHR).

AHR regulates xenobiotic-metabolizing enzymes such as aryl hydrocarbon hydroxylase (cytochrome P450) in humans, mice, rats and neonatal (but not adults) rabbits [18]. AHR over-activation promoted [19,20] while AHR deficiency protected mice from diet-induced obesity [21]. TKP metabolites (Kyn, KYNA and XA) are the endogenous human AHR ligands with potency comparable to exogenous ligands (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin) [22,23]. Aryl hydrocarbon hydroxylase (cytochrome P450) is the major enzyme induced under control of the AHR [18]. Lower hepatic microsomal aryl hydrocarbon hydroxylase and lower nuclear transcription rate of CYP2B1/2B2 mRNA [25] suggested AHR signaling pathways deficiency in ZFR. In this vein, up-regulated formation of KYNA, one of the strongest human hepatic AHR ligands [22], may represent an adaptive response aimed to overcome impairment of AHR signaling pathways in ZFR.

Besides interaction with AHR, elevated KYNA might affect ZFR via antagonism to NMDAR and α7nAChR. It was suggested that enhanced production of KYNA in astrocytes and increased extracellular KYNA inhibit dopamine (DA) release by blocking α7nAChR [26]. Decreased D2 receptor binding and elevated D2/3 receptor availability were reported in obesity [27,28]. Impaired DA function in ZFR was considered to be acquired, rather than inherited, trait caused by circulating factors associated with obesity [29]. Our present data suggest that elevated serum KYNA might be one of such circulating factors. Elevated KYNA was associated with cognitive impairment [30,31], and might underline profound deficits of cognitive functions (e.g., learning and memory), observed in ZFR [32].

Cognitive impairment in schizophrenia might be associated with up-regulated formation of KYNA in brains and CSF of schizophrenia patients [33]. Elevation of serum concentrations of both KYNA and AA was observed in schizophrenia patients as well [34]. Present finding of serum KYNA elevation in ZFR, an experimental model of obesity, suggest a potential role of KYNA in increased prevalence of obesity in schizophrenia patients.

Acknowledgments

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