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Decreased levels of kynurenic acid in the intestinal mucosa of IBS patients: Relation to serotonin and psychological state

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ABSTRACT

Objective: Irritable bowel syndrome (IBS) has been associated with psychiatric comorbidity and alterations in serotonergic metabolism. Tryptophan is the precursor of serotonin (5-HT), but it is mainly catabolized through the kynurenine pathway. This pathway may also be involved in the pathogenesis of IBS by virtue of deviating tryptophan from the 5-HT pathway resulting in 5-HT deficiency. We therefore aimed to ascertain the mucosal and systemic concentrations of 5-HT and kynurenic acid (KYNA), a principal kynurenine metabolite.
Methods: Duodenal mucosal biopsy specimens and platelet poor plasma samples were obtained from 15 healthy volunteers and 15 IBS patients. Psychological state was assessed using the Hospital Anxiety and Depression Scale and the Symptom Checklist-90.
Results: IBS patients showed significantly lower mucosal and higher systemic concentrations of both 5-HT and KYNA compared to healthy controls. Also, significant correlation between mucosal but not plasma concentrations of KYNA and 5-HT and psychological state in IBS was observed.
Conclusion: The observation that mucosal KYNA and 5-HT are both decreased in IBS does not support the hypothesis that increased activation along the kynurenic pathway results in relative 5-HT deficiency. However, an increased release of these substances from the intestine to the systemic compartment may lead to a decrease in intestinal KYNA and 5-HT levels, resulting in disturbance of intestinal homeostasis. Thus, changes in psychological states observed in IBS patients may be secondary to alterations in gastrointestinal function, and in particular kynurenine and/or 5-HT metabolism.

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder affecting up to 15% of the Western population. Despite being very common, the pathophysiology of IBS is incompletely understood [1]. Several studies have provided evidence that IBS is associated with a dysregulation of the brain–gut axis — a theoretical concept describing a bidirectional connection, of which serotonin (5-hydroxytryptamine, 5-HT) appears to be an important modulator [2]. In the periphery, 5-HT is involved in mediating gastrointestinal secretion, motility and perception, whereas in the central nervous system (CNS), 5-HT modulates an extensive range of physiological and behavioral processes [3]. 5-HT is derived from tryptophan. Merely 1% of ingested tryptophan is converted into 5-HT, while the majority is catabolized via the kynurenine pathway, the primary route of tryptophan metabolism in the human body [4]. Metabolites of the kynurenine pathway have recently received increasing attention, as they are also believed to have a regulatory role in both the CNS and the gastrointestinal tract [4,5].

We have recently demonstrated that these two metabolic pathways (5-HT and kynurenine) compete for their mutual precursor tryptophan, in particular following acute stress [6]. This implies that diversion of tryptophan to the kynurenine pathway may potentially lead to a relative deficiency of 5-HT synthesis and hence serotonergic dysfunction. This concept of pathway imbalance has been suggested as a factor involved in the development of disorders associated with serotonergic dysfunction in both CNS and periphery [4,7]. Such a metabolic dysfunction may also provide a mechanistic link between IBS and its high comorbidity with psychiatric conditions such as affective and anxiety disorders. The aim of this study was therefore threefold. First, we aimed to assess the mucosal and systemic concentrations of kynurenic acid (KYNA), a principal kynurenine metabolite, suggested to play a role in gastrointestinal function [8], in IBS patients compared

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to healthy controls. Second, we aimed to assess the mucosal and systemic concentrations of 5-HT, in order to ascertain the relationship between the two pathways. Third, we aimed to ascertain the association between potential alterations in 5-HT and KYNA concentrations with psychological state.

Methods

This study was part of a larger project investigating the role of 5-HT in IBS approved by the Medical Ethics Committee of the Maastricht University Medical Centre (MUMC) and was conducted according to the revised version of the Declaration of Helsinki (October 2008, Seoul). All participants gave their written informed consent prior to participation. The study has been registered at the US National Library of Medicine (http://www.clinicaltrials.gov, NCT00731003).

Participants

Fifteen healthy volunteers and 15 patients with IBS diagnosed according to the Rome III criteria were included. Exclusion criteria included history of gastrointestinal disorders other than IBS; use of psychoactive medication (including serotonin reuptake inhibitors); and use of medication influencing gastrointestinal function within 14 days prior to testing. During the screening period, psychological state was assessed using the 17-item Hospital Anxiety and Depression Scale (HADS) and the Symptom Checklist-90 (SCL-90). Global Severity Index on the SCL-90 was used to assess general psychological state.

Study procedures

Participants arrived at the MUMC after an overnight fast at 8:00 AM. Blood samples were drawn from the antecubital vein. Hereafter, at 10:00 AM, participants underwent a gastroduodenoscopy, during which mucosal samples from the horizontal part of the duodenum were obtained and immediately frozen in liquid nitrogen. The rationale for taking duodenal samples was that this part of the intestine has a high turnover of 5-HT and recent reports suggest an impairment of serotonergic function in the duodenum in IBS [9].

Assessment of kynurenic acid and 5-HT concentrations

Blood samples were collected using pre-chilled K_2EDTA tubes. To avoid oxidative breakdown, 1 ml 1.4% ascorbic acid (Sigma Aldrich, St. Louis, MO) was added to the tubes. Platelet poor plasma samples were obtained by centrifuging tubes at 2000 *g* at 4 °C for 10 min. Supernatants were allocated and immediately frozen at −80 °C until analysis. Biopsy specimens were weighed and homogenized prior to analysis. Concentrations of 5-HT and KYNA were determined by HPLC–MS as described previously [6].

Statistical analyses

The descriptive statistical analyses were performed using SPSS 20.0 for windows (SPSS Inc., Chicago IL). Data were tested for normality by the Kolmogorov–Smirnov test. Normally distributed data were analyzed by Student's t-test. Mann–Whitney U-test was used for non-parametric data. Coefficients for correlations were calculated according to Pearson and Spearmen, respectively. Data are expressed as mean ± SEM, if not otherwise indicated. A Bonferroni correction was applied for multiple testing of correlations with psychiatric scores.

Results

Table 1 describes demographic characteristics and psychological score. Distribution of IBS subtypes was as follows: 46% diarrhea predominant, 33% constipation predominant, 20% mixed subtype.

Table 1
Demographic characteristics and psychological score.

	IBS patients (N = 15)	Healthy controls (N = 15)	p value
Age	44 ± 13	33 ± 17	.06
Gender	46% male	33% male	.35
BMI	27 ± 5	24 ± 4	.08
HADS	3 (0–10)	0 (0–4)	.001
HADS-D	2 (0–6)	0 (0–4)	.007
HADS-A	0 (0–4)	0 (0–0)	.03
Global Severity Index (SCL-90)	117 (91–164)	94 (90–106)	<.001

IBS = irritable bowel syndrome; BMI = body mass index; HADS = Hospital Anxiety and Depression Scale; SCL-90 = Symptom Checklist-90.

Plasma and mucosal KYNA concentrations

Plasma concentration of KYNA was significantly higher in IBS compared to healthy controls (2.96 ± 0.33 vs 0.36 ± 0.019 nmol/l, p < .0001, see Fig. 1A). On the contrary, mucosal concentrations of KYNA were significantly lower in IBS patients compared to healthy controls (0.30 ± 0.10 vs 1.36 ± 0.52 pmol/mg wet tissue, p = .02, see Fig. 1B). A significant negative correlation was found between mucosal and plasma concentrations of KYNA (r = −0.45, p = .03), suggesting an inverse relationship between these two pools of KYNA. No association was found between age, gender or IBS subtype and KYNA concentrations.

Plasma and mucosal concentrations of 5-HT

A similar pattern of alterations was found in IBS with regard to 5-HT concentrations. Plasma concentration of 5-HT was significantly higher in IBS compared to healthy controls (26.2 ± 4.7 vs 1.9 ± 0.35 nmol/l, p < .0001, see Fig. 1C). In contrast, mucosal concentrations of 5-HT were significantly lower in IBS patients compared to healthy controls (12.6 ± 3.4 vs 51.9 ± 17.6 pmol/mg wet tissue, p = .008, see Fig. 1D). On the contrary, a significant negative correlation was found between mucosal and plasma concentrations of 5-HT (r = −0.42, p < .05). No association was found between age, gender or IBS subtype and 5-HT concentrations.

Correlation between 5-HT and KYNA concentrations

We also investigated whether there was any relation between levels of 5-HT and KYNA. We found a significant negative correlation between plasma 5-HT and KYNA concentrations both in healthy controls (r = −0.60, p = .03) and in IBS patients (r = −0.62, p = .02), suggesting an inverse relation. On the other hand, no correlation was found between mucosal concentrations of 5-HT and KYNA in either group (data not shown).

Correlations with psychological score

In IBS patients, a significant correlation was found between mucosal 5-HT and HADS score (r = 0.66, p = .02) but not with the SCL-90 score (r = 0.15, p = .64). When subdividing the HADS score into domains of depression and anxiety, mucosal 5-HT concentrations significantly correlated with the HADS-D score (r = 0.74, p = .006) but not with HADS-A score (r = 0.25, p = .42). Furthermore, a significant negative correlation was found between mucosal KYNA concentration and SCL-90 (r = −0.57, p < .05) but no correlation was found with the HADS score (r = −0.34, p = .27). In contrast to mucosal 5-HT and KYNA, no correlations were found for plasma 5-HT and KYNA concentrations and HADS or SCL-90 in IBS. It is important to note that following Bonferroni correction for multiple testing, only correlation between mucosal 5-HT and HADS-D score remained significant.

In healthy controls, no correlation was found between HADS scores and SCL-90 and either plasma or mucosal 5-HT concentrations or plasma or mucosal KYNA concentrations (data not shown).

Discussion

A number of studies have found evidence for a dysregulation in the kynurenine pathway in IBS. The present study, although limited in sample size, is the first to report on both mucosal and systemic levels of KYNA. We found decreased mucosal levels and increased systemic levels of KYNA in IBS patients. KYNA is an antagonist at the N-methyl-D-aspartate and nicotinic cholinoreceptors and an agonist at the orphan G-protein-coupled receptor GPR35, at which KYNA is one of the most potent endogenous agonists yet identified [10]. GPR35 has been shown to have anti-nociceptive properties and has therefore been suggested as an important target to counteract inflammatory pain [11]. The highest levels of GPR35 are in the intestine, and

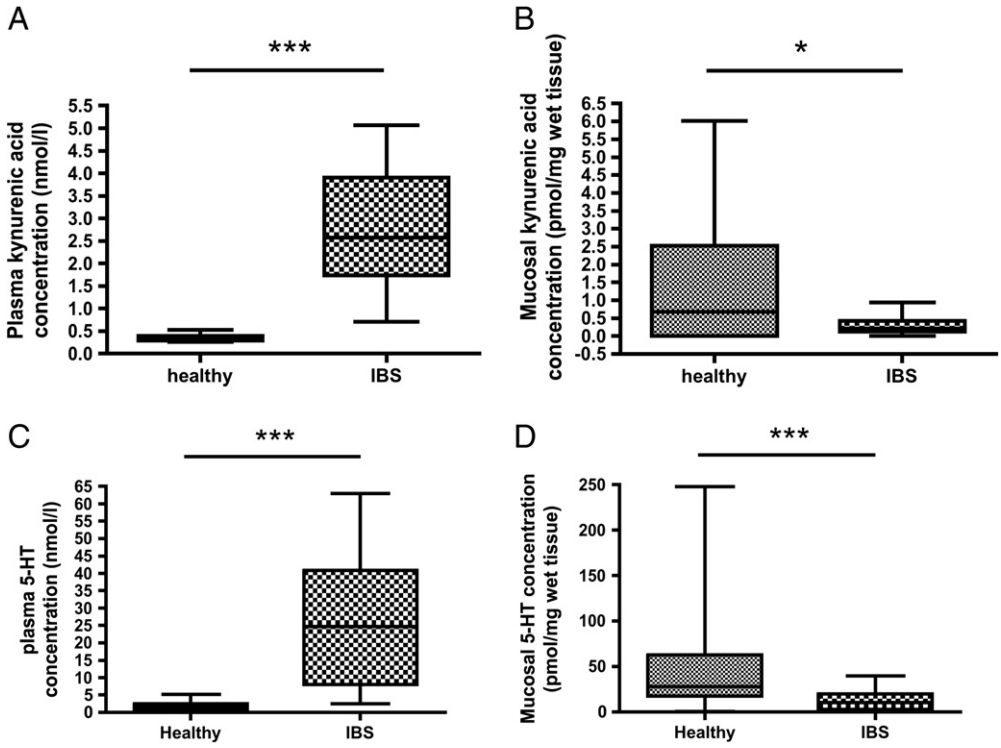

Fig. 1. Plasma and mucosal concentrations of kynurenic acid and serotonin (5-HT) in patients with irritable bowel syndrome (IBS) and healthy controls. *p<.05; ***p<.001.

thus GPR35 may play an important role in mediating the actions of KYNA [12]. In the intestine, KYNA is known to have neuroprotective, anti-oxidative and anti-inflammatory properties and is believed to play a role in gut motility and sensory functions [13]. Decreased mucosal concentrations of KYNA may therefore potentially contribute to functional, neural, metabolic or inflammatory derangements that facilitate the development of gastrointestinal disorders, such as IBS.

Interestingly, we found increased plasma levels of KYNA in IBS, although it is important to note that findings in literature are not consistent [14–16]. Earlier studies showing increased plasma levels of kynurenic acid in patients with inflammatory bowel disease suggested that this increase may represent either a compensatory response to elevated activation of enteric neurons or a primary abnormality which induces a compensatory increase in gut activity [17]. Systemic levels of kynurenine metabolites are largely under control of the liver by clearing any excess tryptophan through tryptophan dioxygenase (TDO), the initiating enzyme of the kynurenine pathway. Another source of kynurenine metabolites in plasma is derived from the activity of extrahepatic indoleamine-2,3-dioxygenase, largely expressed by the intestine [4]. KYNA is synthesized from kynurenine by action of kynurenine aminotransferase, expressed primarily in the liver, intestine and kidney [18]. The source of increased plasma KYNA concentrations is therefore most probably peripheral, as KYNA only poorly penetrates the blood–brain barrier [19]. Increased systemic concentrations of KYNA may reflect either 1) increased activity of hepatic TDO or 2) increased release of KYNA from extrahepatic sources, such as the intestine. The former seems less likely since previous reports have suggested decreased TDO activity in IBS [15]. The latter, however, is supported by the inverse relationship between intestinal mucosal and systemic levels of KYNA observed in our study. An increased release of KYNA from the mucosa into to systemic compartment could therefore result in mucosal KYNA deficiency, potentially contributing to intestinal dysfunction.

We also assessed 5-HT concentrations to ascertain their relation to KYNA concentrations. Levels of 5-HT in both plasma and mucosa showed a pattern similar to KYNA levels in IBS: significantly elevated plasma concentrations and decreased mucosal concentrations compared to healthy controls. Previous studies in IBS have also demonstrated higher plasma 5-HT levels [20] and lower 5-HT levels in mucosa of IBS patients [21], although it is noteworthy that considerable discrepancies still exist in data from various reports [22].

Plasma concentrations of 5-HT and KYNA were found to be inversely related in our study. However, the observation that plasma KYNA and 5-HT are both increased in IBS does not support the hypothesis that increased activation along the kynurenic pathway results in relative 5-HT deficiency contributing to disease development. Furthermore, no correlation was found in mucosal concentrations of 5-HT and KYNA. It is important to note, that we measured only a single metabolite of the kynurenine pathway, which is not necessarily representative of the entire spectrum of kynurenine metabolites. Thus, it cannot be excluded that both alterations in 5-HT and kynurenine metabolism play a role in IBS, regardless of whether these changes are interdependent. A more complete profiling of mucosal and systemic concentrations of these metabolites should therefore add to our understanding of the role of the kynurenine pathway in IBS.

We also ascertained whether these changes in 5-HT and KYNA concentrations were associated with psychological state. We found no correlation between plasma concentration of 5-HT or KYNA and HADS or SCL-90 in IBS, which is in line with the findings of Park et al. [23]. However, more interestingly, we observed a significant correlation between mucosal concentrations of 5-HT and psychological state in IBS patients. Apart from the disturbances in the 5-HT pathway, kynurenic pathway dysregulation has previously been associated with affective disorders [24]. Plasma levels of kynurenic acid have either been shown to be decreased [25] or unchanged in this patient group [26]. Patients with depression (without gastrointestinal comorbidity) generally also have lowered plasma levels of 5-HT [24,27]. Considering the increased plasma levels of 5-HT and KYNA in IBS and the lack of correlation with psychological scores, we postulate that the changes in psychological state observed in IBS patients in our study may be secondary to alterations in gastrointestinal function, and in particular kynurenine and/or 5-HT metabolism. This is an important assumption

since IBS patients have been shown to be hypervigilant regarding gastrointestinal symptoms [28]. Potential alterations in gastrointestinal homeostasis may therefore be augmented by hypervigilance and contribute to increased disease burden. Consistent with this hypothesis is that cognitive behavioral therapy only indirectly improves bowel symptoms through improvement of mood and anxiety [29].

Due to the preliminary nature of our findings and the relatively small sample size, further research is warranted to confirm the formulated hypotheses. It is also important to note that no differentiation was made with regard to different IBS subtypes. Furthermore, correlations with psychological scale should be interpreted with caution, as the small sample size and stringent multiple hypothesis corrections are factors possibly causing false negatives results. Findings of this study will therefore need to be substantiated in a larger group of patients.

Disclosures

The authors have no competing interests to report.

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Author contributions

DK designed the study, analyzed the data and wrote the manuscript, FT and DM were involved in the study design and supervision, JK and CL provided expert opinion and reviewed the manuscript, and AM was the overall supervisor of the study.

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