

Sex differences of brain serotonin synthesis in patients with irritable bowel syndrome using α -[^{11}C]methyl-L-tryptophan, positron emission tomography and statistical parametric mapping

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BACKGROUND: Irritable bowel syndrome (IBS) is the most common functional bowel disorder and has a strong predominance in women. Recent data suggest that the brain may play an important role in the pathophysiology of IBS in the brain-gut axis. It is strongly suspected that serotonin (5-HT), a neurotransmitter found in the brain and gut, may be related to the pathophysiology of IBS. It is reported that a 5-HT₃ antagonist is effective only in female patients with diarrhea-predominant IBS.

OBJECTIVE: In the present study, 5-HT synthesis was measured using positron emission tomography, with α -[^{11}C]methyl-L-tryptophan as the tracer, in patients with IBS. The aim of the present study was to compare 5-HT synthesis in the IBS patients with that in the controls, and to compare 5-HT synthesis between male and female IBS patients.

METHODS: Six male and six female nonconstipated IBS patients were scanned. Age-matched healthy volunteers were scanned as controls. Eighty minute dynamic scans were performed. Functional 5-HT synthesis images were analyzed using statistical parametric mapping.

RESULTS: 5-HT synthesis was greater only in the female IBS patients in the right medial temporal gyrus (multimodal sensory association cortex) compared with the female controls ($P < 0.001$).

CONCLUSIONS: The greater brain 5-HT synthesis in the female IBS patients than in the controls may be related to the pathological visceral pain processing of the IBS patients, a larger female predominance of the disorder, and the sex difference of the efficacy of the 5-HT₃ antagonist in treatment.

Key Words: *Irritable bowel syndrome; Positron emission tomography; Serotonin; Sex differences*

Irritable bowel syndrome (IBS) is a common functional bowel disorder, and is characterized by abdominal pain and alterations of defecation (1,2) in the absence of biochemical or structural abnormalities that could normally explain the symptoms. The adult prevalence of the disorder is approximately 20% in western populations (3). IBS is described by multiple factors such as affective factors, including cognitive, and phys-

Différences de sexe quant à la synthèse de la sérotonine dans le cerveau chez des patients souffrant du syndrome du côlon irritable, relevées à la tomographie par émission de positrons avec l' α -[^{11}C]méthyl-L-tryptophane et à la cartographie paramétrique statistique

CONTEXTE : Le syndrome du côlon irritable (SCI) est le trouble fonctionnel intestinal le plus fréquent et il se rencontre surtout chez les femmes. Selon des données récentes, le cerveau jouerait un rôle important dans la physiopathologie du syndrome le long de l'axe cerveau-tube digestif. On croit fermement que la sérotonine (5-HT), un neurotransmetteur présent à la fois dans le cerveau et le tube digestif, n'est pas étrangère à la physiopathologie de l'affection. Des rapports indiquent que l'antagoniste des récepteurs 5-HT₃ n'est efficace que chez les femmes souffrant du SCI à prédominance diarrhéique.

OBJECTIF : Nous avons mesuré, dans la présente étude, la synthèse de la 5-HT au moyen de la tomographie par émission de positrons en utilisant l' α -[^{11}C]méthyl-L-tryptophane comme traceur chez des patients atteints du SCI. L'étude visait à comparer la synthèse de la 5-HT chez des patients souffrant du syndrome et chez des témoins ainsi qu'entre patients de sexe différent.

MÉTHODE : Six hommes et six femmes atteints du SCI sans constipation ont été soumis à la tomographie, de même que des témoins volontaires en bonne santé, appariés selon l'âge. Nous avons d'abord procédé à un enregistrement dynamique de données durant 80 minutes, puis analysé les images fonctionnelles de la synthèse de la 5-HT au moyen de la cartographie paramétrique statistique.

RÉSULTATS : Seules les femmes souffrant du SCI ont présenté une augmentation de la synthèse de la 5-HT dans la deuxième circonvolution temporale droite (aire des associations plurisensorielles) par rapport aux femmes témoins ($P < 0,001$).

CONCLUSION : La synthèse accrue de la 5-HT cérébrale chez les femmes souffrant du SCI comparativement aux témoins peut être liée au processus pathologique de douleur viscérale, trouble à forte prédominance féminine, et à la différence d'efficacité du traitement à l'antagoniste des récepteurs 5-HT₃.

iological and behavioural factors. In addition, all of these different factors are interconnected, making it even harder to describe the definitive importance of each of them in the generation and/or as a consequence of IBS symptoms. The central nervous system modulates peripheral intestinal motor or sensory activity and vice versa. These domains interact through the bidirectional parallel circuits, the so-called brain-gut axis

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which links the visceral afferent sensation and intestinal motor function with higher cortical centres (4-7). Recent advances in the investigation of the brain-gut axis suggest that the brain plays an important role in the pathophysiology of IBS (4-7). For example, stress often induces or exacerbates major symptoms in IBS patients (8,9). IBS patients also show psychoneurotic symptoms and extracolonic somatic symptoms (10). These observations support the hypothesis that not only the gut but also the brain display a dysfunction and exaggerated response to pathophysiological stimuli in IBS subjects. Because there are few animal models of IBS, the study of the brain's involvement in IBS is greatly hampered because all of the studies relating to the peripheral and central components must be performed on humans.

Serotonin (5-HT) is one of the neurotransmitters found in the brain and gut, that may regulate and/or modulate activities related and/or resulting from IBS (11). 5-HT terminals are widely distributed throughout the brain from the cell bodies found in the brainstem (12). Because of the widely spread distribution of 5-HT projections, it is not surprising that serotonergic neurotransmission is involved in many physiological as well as pathophysiological processes in the brain. In addition, there is a great interconnection between many brain structures, which suggests the possibility that 5-HT synthesis in some brain structures may be affected directly by brain-gut interactions, while in other structures, the effect could be an indirect one. Indeed, it has been reported that in diarrhea-predominant IBS patients, postprandially, the serum levels of 5-HT are higher than in the normal population (13). However, it is difficult to relate the plasma levels of 5-HT or 5-HT metabolites to any actions of 5-HT in the brain (14).

It has also been reported that alosetron, a 5-HT₃ antagonist, may have therapeutic potential in female diarrhea-predominant IBS patients (15,16). The 5-HT₃ receptors are present on the peripheral and central neurons (17-19). Although the distributions of the 5-HT₃ receptor in the brain are varied between species, 5-HT₃ receptor sites in humans have been reported to be distributed both to the hindbrain and to the forebrain. In humans, the highest concentration of 5-HT₃ receptors is within the dorsal vagal complex in the brain stem (20). In addition, 5-HT₃ sites are found in the hippocampus, amygdala and the superficial layer of the cerebral cortex. There is also a relatively high level of 5-HT₃ receptors in the human caudate nucleus and putamen. Further, 5-HT₃ antagonists not only have peripheral effects but also central effects, including anxiolytic, pain processing, attenuation of memory impairment and antipsychotic effects (21-23). Thus, from all of this, it can be hypothesized that brain serotonergic neurotransmission and brain 5-HT synthesis may play one of the important roles in the pathophysiology of IBS (11).

It has been reported, in community samples, that IBS symptoms have a two to one predominance in women (3). Recent advances in neuroimaging techniques revealed that patients with IBS may exhibit abnormal integration of painful stimulation centrally (24,25), and in a sex specific manner (26). A sex difference in alosetron serum concentrations (30% to 50% higher in women) has been observed (22), but this difference in pharmacokinetic profiles between the sexes is not thought to be responsible solely for the differences in efficacy (11). Thus, the mechanism of this sex difference is still unclear (11,22).

Alpha-[¹¹C]methyl-L-tryptophan (α -[¹¹C]MTrp) has been developed as a tracer for the measurement of brain 5-HT synthesis rates in living mammals (14). This tracer has been used to measure 5-HT synthesis rates in the human brain using positron emission tomography (PET). The synthesis rates are calculated from the blood-to-brain clearance constant. The method is based on the unidirectional uptake of α -[¹¹C]MTrp, which is transported and, in part, trapped in the brain. It has been shown that in the rat brain, it is converted, in part, to α -methyl-5-HT, and that the brain trapping of α -MTrp correlates with the brain conversion of tryptophan to 5-HT but not with the tryptophan incorporation into proteins or tryptophan transport through the blood brain barrier (14). Tissue radioactivity images of α -[¹¹C]MTrp can be easily converted to functional images representing the regional rates of 5-HT synthesis in the brain using the Patlak plot approach (27). The functional images can then be used for statistical comparisons, such as statistical parametric mapping (SPM). SPM analyses have the advantage over the region of interest comparisons because they remove the intersubject variability, and standardize the images to the Talairach space (28) – the procedures that facilitate comparisons of regional differences. In addition, the observer cannot introduce any bias to the comparison. The authors previously reported on the use of SPM for comparisons between male and female normal subjects (29) as well as between patients with psychiatric disorders, such as borderline personality disorder (30).

In the present study, brain 5-HT synthesis was measured with PET using α -[¹¹C]MTrp as the tracer in 12 patients with IBS, and compared with respective age-matched healthy controls using SPM. On the basis of the possible involvement of 5-HT in IBS, as described above, it was hypothesized that there may be regional differences in brain 5-HT synthesis between IBS patients and the controls; and that there may be sex specific differences in brain 5-HT synthesis among the IBS patients. It could also be hypothesized that these differences in 5-HT synthesis may be related to the brain regions involved in the processing of visceral sensation in IBS patients.

METHODS

Subjects

Six male (mean age 44±9 years; mean ± SD) and six female (mean age 52±11 years) nonconstipation-predominant IBS patients were scanned. The diagnoses of IBS were made based on the Rome I criteria for IBS and each patient underwent a basic evaluation to exclude organic disease. Psychiatric evaluations were also conducted using the *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-III-R (31). The patients did not take any prohibited medication, including IBS treatments, antidepressants, anxiolytics, narcotics, 5-HT₃ antagonists and tryptophan supplements, for more than seven days before the PET scan. Age-matched male (n=6, mean age of 40±13 years) and female (n=7, mean age 52±9 years), right-handed, healthy volunteers were scanned as controls. There is no statistical significance in age between the control and patient and sex groups. Exclusion criteria for the subjects included evidence of a past or present Axis-I or II DSM-III-R diagnosis in the subjects or in the subjects' first-degree relatives, or any significant medical illness, including gastrointestinal diseases.

The study was approved by the Research and Ethics Committee of the Montreal Neurological Institute and Hospital,

McGill University, and Centre Hospitalier de l'Université de Montréal. All the subjects provided written informed consents before the study.

PET procedure

The radiopharmaceutical α -[^{11}C]MTrp was prepared by the method reported by Mzengeza et al (32). The day before the PET study, all subjects received a standard low-protein diet to reduce the variability in the plasma concentrations of amino acids, including tryptophan, that may be attributable to individual differences in diet. The tracer was injected over 2 min, and 80-min dynamic PET scans were performed commencing at the beginning of the tracer injection. The duration of the acquisition frames progressively increased from 1 to 10 min. Blood samples drawn from the antecubital vein were taken at progressively increased time intervals (15 s to 10 min). Five additional venous samples were drawn during the PET scans for high performance liquid chromatography analysis to measure the free and total plasma concentration of tryptophan. The PET scans were obtained with an ECAT EXACT HR+ whole-body tomography scanner (CTI/Siemens, USA) that has 63 image slices at an intrinsic resolution of 5.0 \times 5.0 \times 5.0 mm full-width half-maximum value. The scans were acquired and reconstructed three dimensionally. The images were blurred, for use with SPM, with a Gaussian filter to a final resolution of 14 mm. The transmission scans were performed using ^{68}Ga for the attenuation correction.

Each subject underwent a high resolution magnetic resonance imaging (MRI) scan (160 slices, 1 mm thick) obtained with a Siemens Vision (1.5T) (CTI/Siemens, Mississauga, Ontario). The coregistration of the individual PET and MRI images was performed using an automatic procedure (33). The MRI images from each subject were transferred into Talairach space (28) and coregistered with the functional PET images (34). Comparisons between groups were done using the SPM program.

To calculate 5-HT synthesis rates with the venous samples, normalized input functions were estimated, using a previously reported procedure (35). Briefly, the input function for the initial 20 min was derived from time-radioactivity curves (TACs) obtained from the sagittal sinus normalized to the venous plasma radioactivity at 20 min. After 20 min, the venous plasma TAC was used. TACs from the sagittal venous sinus were established by drawing small regions of interest on two to three slices at the level of the confluence of sinuses. Calculations of 5-HT synthesis rate images were then made with normalized venous sinus TACs derived from a combination of the two curves. As was previously reported, this approach provides results not significantly different from those obtained using the arterial input function (34,35).

The rate of 5-HT synthesis (R , pmol/g/min) is an algebraic product of the net blood to brain clearance of the tracer (K^* , mL/g/min) and the availability of the endogenous 5-HT precursor, L-tryptophan (14,36,37). To calculate K^* , tissue data, in the form of distribution volumes (mL/g), were fitted as a function of exposure time (min). K^* values were then determined using the Patlak plot method (27) applied to the data points obtained from 20 min to 60 min as previously reported (29,34,35). Functional images of K^* were then calculated pixel by pixel, and these were converted into images of 5-HT synthesis rates using the equation $R=K^* \cdot C_p^{\text{Free-Trp}} \cdot \text{LC}^{-1}$, where $C_p^{\text{Free-Trp}}$ stands for the concentration of plasma free tryptophan. A lumped constant (LC) of 0.42, measured in vivo in the rat brain (38), was used to convert the tissue uptake constant of α -MTrp into the uptake constant of trypto-

phan. The latter constant, multiplied by the concentration of plasma free tryptophan, provides the 5-HT synthesis rate. Differences between the LC in rats and humans are possible, but they would not have any effect on the between-group comparisons. Within a given species, the LC is assumed to be constant. It should be emphasized that any comparisons done with SPM using proportional scaling are not sensitive to the individual or group differences in the LC. In addition, when proportional scaling is used, there is no need to calculate the functional images of 5-HT synthesis rates because the SPM analysis results are identical to those obtained with K^* functional images.

Statistical analysis

Plasma free and total tryptophan were compared statistically by an analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$.

The functional PET data was analyzed with SPM analysis, using the SPM99 software (39-42). Before the SPM comparison, all of the images were smoothed with the Gaussian filter (see above) to increase the signal-to-noise ratio and to reduce the effect of the individual variability in the cortical gyral anatomy of the brain (39-41). The functional images were normalized using proportional scaling to the global mean (global mean = 50 pmol/g/min; close to the mean 5-HT synthesis rate or in the case of K^* – images to 10 mL/g/min). SPM comparisons were performed at a threshold of 80% of the peak value (39). The t -test was applied pixel by pixel to compare the regional differences in 5-HT synthesis images between the men and women. The SPM(t) data for each pixel was then transformed to the unit normal distribution (SPM(z)), which makes parametric maps independent of the effective degrees of freedom (40). This constituted statistical parametric maps. Two thresholds were used: the height threshold (u) of $P < 0.001$, and the extent threshold (k) of 100 voxels (voxel 2 \times 2 \times 2 mm) was used to remove small clusters resulting from noisy voxels (42). The significance of each cluster was assessed according to the procedure suggested and described by Friston et al (40). The conversion of the probability maps to the z -scores in which the probabilities are represented by the location on a Gaussian distribution of each probability makes the statistical results independent of the effective degrees of freedom of the data set (41). Because distribution is independent of the effective degrees of freedom, in SPM(z), and there has been limited data obtained using SPM analysis for 5-HT synthesis functional images, it was decided to report the clusters with $P < 0.001$ without correction for multiple comparisons, as proposed by Friston et al (40). The cluster size of 100 voxels would exclude some small structures such as the dorsal raphe, but until more data with α -[^{11}C]MTrp are obtained, smaller cluster size cannot be justified.

RESULTS

In Table 1, the plasma total and free tryptophan concentrations in the male and female controls, and the IBS patients are provided. There was a significant difference ($P < 0.05$) in the plasma total tryptophan concentration between the male IBS patients and the male control subjects, and in the plasma free tryptophan concentration between the female IBS patients and the female controls. The free tryptophan concentration of female controls was higher than that of the female IBS patients.

The SPM comparison between the male and female healthy controls did not have any significant differences in brain 5-HT synthesis when the above mentioned criteria were used (a clus-

TABLE 1
Plasma total and free tryptophan concentrations in controls and irritable bowel syndrome (IBS) patients

	Total tryptophan (nmol/mL)	Free tryptophan (nmol/mL)
Control subjects		
Male	94±29 [†]	11.3±5.8
Female	53±14	10.7±1.6 [‡]
IBS patients		
Male	52±6.8	9.6±3.3
Female	50±17	7.1±2.0

Data are presented as mean ± SD. [†]The plasma total tryptophan concentration in the male controls was significantly higher than that in the female controls as well as in the male and female IBS patients; [‡]The plasma free tryptophan concentration in the female controls was significantly higher than in the female IBS patients

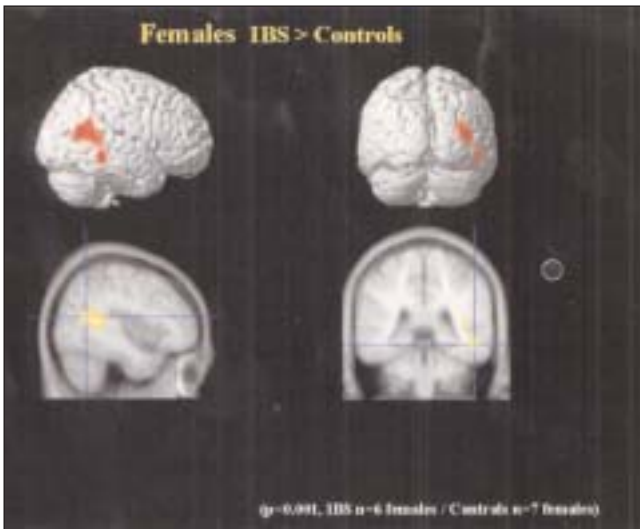


Figure 1 A set of images exemplifying statistical parametric mapping analysis in which serotonin (5-HT) synthesis in the female irritable bowel syndrome (IBS) patients was compared with that in the age-matched female controls. The areas in which 5-HT synthesis was found to be significantly higher in the female IBS patients than in the female controls are superimposed on the three dimensional images of the Talairach brain. The cross sections through the brain, overlaid to magnetic resonance images, are also shown in areas in which the synthesis significantly differs ($P < 0.001$)

ter of 100 voxels and a height at $P < 0.001$). There were also no clusters reaching these criteria for significance between the different sexes in the IBS group (data not shown).

In the SPM analysis of the data sets after separating the subjects into sex specific groups, we only observed significant differences in brain 5-HT synthesis in the female comparisons. The brain areas with significant differences in 5-HT synthesis that are superimposed on the three dimensional images of the Talairach brain or overlaid on the representative MRI slices are shown in Figure 1. In Table 2, the clusters identified by the SPM analysis in the comparison of the female controls and the female IBS patients are provided. 5-HT synthesis was greater in the female IBS patients in the right medial temporal gyrus (multimodal sensory association cortex) than in the female controls.

In contrast to the results of the comparison between the female patients, there is no significant difference between male

TABLE 2
Brain regions showing significantly ($P < 0.001$) higher serotonin synthesis in the female irritable bowel syndrome patients ($n=6$) than in the age-matched female controls ($n=7$)*

Brain region	Coordinates				
	BA	Z	x	y	z
R medial temporal gyrus	21	3.81	52	-38	-12
R medial temporal gyrus	39	3.73	42	-58	14
R medial temporal gyrus	39	3.70	42	-48	10
R medial temporal gyrus	39	3.67	38	-66	12

*The clusters are identified by anatomical name and Brodmann area (BA) identification, as well as the Z-value and the stereotaxic Talairach coordinates (x,y,z [mm]). The Z-values relate to the normal distribution. R Right

IBS patients and the age-matched male control subjects. In a comparison between all the IBS patients (males and females pooled together) and the pooled control subjects, there was no significant brain region in which 5-HT synthesis differed between the pooled control subjects and the pooled IBS patients at this threshold.

DISCUSSION

In the present study, we demonstrated for the first time that there are significant differences in regional 5-HT synthesis in the brain between female IBS patients and female controls. This is the first study evaluating 5-HT synthesis in IBS patients and it possibly indicates that brain-gut interactions are related to 5-HT synthesis in such patients. There was no significant difference between the male patients and the respective controls.

The plasma total tryptophan in the male controls was significantly higher than that in the female controls, and also higher than in the male and female IBS patients. The plasma free tryptophan in the female controls was significantly higher than that in the female IBS patients. Lower plasma total and free tryptophan levels in the women than in the men were observed in our previous study (44) and in studies reported previously by others (45,46). Several investigators have shown that plasma free Trp correlates the best with brain tryptophan and brain 5-HT synthesis in both laboratory animals (47) and humans (48). However, it should be noted that the differences in the plasma tryptophan do not have any effect on the findings with SPM, when using proportional scaling. The proportional scaling removes these differences through normalization, because even if the plasma total Trp influences 5-HT synthesis (note that the contrary has been shown in many studies (14) it would result in a global increase in the synthesis that, after the proportional scaling, would be removed. However, the proposition that the plasma total tryptophan could have regional specific influences on 5-HT synthesis is rather unlikely. There is no clear explanation of the reasons for these differences in the plasma total tryptophan. We previously reported on a large variability and the differences in absolute rates of 5-HT synthesis between men and women using the region of interest analysis and different PET scanners (29,34,35), as well as some regional differences between men and women using SPM analysis. However, in that study we used less stringent criteria for significance (29) than in the present study. The use of less stringent criteria is likely the reason why there was no significant sex difference in 5-HT synthesis in the present control group.

In sex specific comparisons of SPM, 5-HT synthesis was higher in the multimodal sensory association cortex in the female IBS patients only when they were compared with their sex-matched controls. In contrast to extensive literature on somatic pain, there are few reported studies of sex differences in visceral pain perception (49,50). Recently, sex differences in the regional cerebral blood flow response to visceral stimuli in IBS patients using PET were reported (26). In this study, it was reported that there is greater insula activation by a visceral stimulus in male than in female IBS patients (26). In our study, with no visceral stimulation, there was no increased 5-HT synthesis in the insula, anterior cingulate gyrus, thalamus, prefrontal cortex or cerebellum, as seen in that study (26). The differences between our results and the results of the former study may be caused by differences in the protocol (with versus without rectal stimuli) and different biological and physiological parameters that were measured (regional cerebral blood flow versus 5-HT synthesis). Our study is aimed at evaluating the brain 5-HT differences in vivo in IBS patients. In other words, we aimed to demonstrate the relationship between chronic IBS symptoms itself and the brain 5-HT synthesis, without any manipulation or stimulation. It has been reported, however, that the connection from the multimodal sensory association cortex to the prefrontal cortex and the limbic system go through the cingulate gyrus, parahippocampus and insula (51). The prefrontal cortex is responsible for cognition. The multimodal convergence in the medial temporal cortex has the pivotal role as the final and supreme site of integration for all aspects of mental function (52). The importance of this serial processing and multimodal convergence to cognitive function was widely accepted (52).

In contrast to the comparisons between female patients and female controls, there are no significant differences in the male comparisons and in the comparisons between the male and female IBS patients in this study. This may be due to the small

sample size and/or strict statistical threshold we used. With a less stringent threshold at $P < 0.005$ and 100 voxels, however, the male IBS patients also showed higher 5-HT synthesis than that of the age-matched controls in the multimodal sensation association cortex, although the significance and size of clusters are less and smaller than the female comparisons with the same threshold (data not shown). Further, at this threshold, the female IBS patients also showed higher 5-HT synthesis in the prefrontal cortex than the female controls (data not shown). Everyday experiences, including sensory information, unfold in multiple modalities (52). Chronic symptoms of IBS, including visceral sensation through the neuronal network, which is mentioned above, could affect the multimodal convergence from sensation to cognition in this cortex, as the brain-gut axis. The results of our study may suggest the important role of this cortex in the pathophysiology of IBS and may provide an explanation of the central effects of alosetron in IBS through the 5-HT₃ antagonism in the multimodal sensation association cortex with sex differences.

CONCLUSIONS

Our preliminary data presented here suggest that chronic IBS symptoms result in an increase in 5-HT synthesis in the brain, and these increases appear to be sex specific. The higher 5-HT synthesis in the brains of female IBS patients than in the controls may be related to the pathological visceral pain processing of IBS patients, a larger predominance and the efficacy of the 5-HT₃ antagonist in women.

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