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Original Article

Serotonin (5-HT), tryptophan and 5-HIAA in weanling male albino rats fed with different local carbohydrate diets in Enugu, South-East Nigeria.

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A R T I C L E I N F O

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A B S T R A C T

The mammalian brain has been known to respond to both acute and chronic changes in diet and nutritional research has focused mainly on the effects of long-term, dietary manipulation on the brain. The present study was designed to determine the effect of different local carbohydrate (CHO) diets in Enugu, Nigeria, on plasma and brain tryptophan and brain serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) metabolism, in male albino wistar rats. Twenty (20) weanling male albino wistar rats weighing between 80-85 grams were divided into four groups (A-D) of five rats each and allowed to acclimatize. They were fed on balanced diet containing the same quantity (73.75g) of garri, rice, yam and sucrose (control) respectively, in a 100g diet for two weeks. The body and brain weights were obtained and the concentrations of blood sugar, plasma and brain tryptophan (TRP), whole brain protein, 5-HT and 5-HIAA were determined using standard analytic methods. Data obtained was analysed by student's t-test at 99% confidence interval using SPSS computer software package and the results presented as mean ± SEM. The results showed that yam significantly increased (P<0.001) brain 5-HT metabolism whereas rice significantly increased body and brain weights and also blood sugar concentrations (P<0.05). However garri showed significantly lowered values (P<0.05) in all the measured parameters.Local CHO influence serotonin metabolism. Thus in diet therapy, yam should be the preferable dietary CHO for conditions involving diminished 5-HT release. Similarly, garri should be considered as the CHO of choice in individuals requiring CHO restriction to lower blood sugar, especially in diabetics.

1. Introduction

Nutritional research has focused mainly on the effects of long-term, dietary manipulation on the brain, because chronic malnutrition has been shown to be associated with well-known disease states [1]. The particular brain constituent known to increase, depending on the food consumed are the neurotransmitter, serotonin (5-hydroxytryptamine - 5HT) and its precursor amino acid, tryptophan (TRP) [2]. The rate of brain serotonin synthesis normally depends on its concentration of TRP, serotonin's essential amino acid precursor [1-4]. This is because tryptophan hydroxylase (EC 1.13.99.3), the enzyme that catalyzes the initial and rate-limiting step, has a very low affinity for TRP and is thus highly unsaturated at physiologic brain TRP concentrations [5]. Brain TRP concentrations and the flux of TRP from blood to brain, depend, in turn, partly on plasma TRP and partly on plasma concentrations of 6 other large neutral amino acids (LNAAs) or branch chain neutral amino acids (BCAA): tyrosine, phenylalanine, leucine, isoleucine, valine, and methionine [2, 6, 7], which compete with TRP for blood-brain barrier transport [8]. Because dietary carbohydrates and proteins affect plasma concentrations of TRP and the other BCAAs [9, 10], these macronutrients also affect brain TRP concentrations and, thereby, serotonin synthesis and release [11, 12]. It has been shown that ingestion of single meal readily alters blood BCAA levels [13]. These amino acids like valine, isoleucine and Leucine compete with TRP for the same transport site in the brain and predictably, may influence brain TRP levels

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and 5-HT biosynthesis [14]. Carbohydrate (CHO) ingestion causes insulin secretion, which lowers blood levels of these BCAAs. As a consequence, blood TRP that is little affected by this mechanism is increased [15]. This gives blood TRP a competitive advantage over the other BCAA and thus its enhanced uptake (tryptophan-sparing effect), leading to increased brain 5-HT content [13]. Useful clinical applications of TRP and possible use of specific means that would increase brain TRP levels have been identified [14]. The advantage of this is the stimulation of 5-HT formation. For instance, TRP administered to rats is known to stimulate growth hormone secretion [15], produce analgesic effect [16], to lower blood pressure [17] and to reduce CHO consumption in animals allowed to select their own intake of proteins and CHO [15]. A variety of drugs used in neuropharmacology and psychopharmacology act by modifying brain neurotransmitter levels and synthesis. Some of these have been shown to be influenced by prior food consumption [18]. CHO consumption or TRP administration can also modulate some normal behaviours like increasing fatigue and sleepiness, accelerating sleep onset in people with prolonged sleep latencies [19]. Although evidence exists that synthetic-formula meals can actually affect the plasma tryptophan ratio [9, 10, 16], producing two-fold differences, depending on their proportions of carbohydrates and proteins [16], few data are available on the changes in this ratio produced by the kinds of meals (local diets) that Africans normally eat, especially in Nigeria. Hence, we examined the changes in the body and brain weights, blood glucose, plasma and brain TRP, brain protein, 5-HT, 5-HIAA, that result from consuming three relatively typical local carbohydrate diets (Table 1), one containing yam (73.75 g) in a hundred grams meal, the second containing rice (pre-boiled) (73.75 g) in a hundred grams diet and the third containing garri (processed cassava grains) fried (73.75 g) also in a hundred grams diet. Since human brain tissues are difficult to obtain and experimentation in-vivo in humans is ethically unacceptable, most information can be extrapolated from studies on experimental animals, especially rats, which were used in the present study. Apparently no data are available on the concurrent effects of specific macronutrients on serotonin and catecholamine release in the brains of experimental animals [20].

The general objective of this study is to assess the extent to which different local CHO diets influence the rate at which the brain neurons take up TRP for the synthesis of 5-HT in experimental animals. This could offer an advantage for the dietary treatment of diseases states involving diminished release of this neurotransmitter, like insomnia and depression and enhanced management of conditions like diabetes. The study may also throw more light on psychiatric-derived complications.

2. Materials and Methods

2.1. Experimental Animals

Twenty male albino wistar rats from the animal house unit of the department of Pharmacology and therapeutics and weighing between eighty and eighty-five grams were used throughout the study. The animals were housed in groups of five in standardized cages made of galvanized metal, with a galvanized wire mesh base. The animals were divided into four groups (A-D) with the fourth group (group D) serving as the control. The animals in each group. were closely matched for body weight and were distinguished from each other by markings round the tail. All animals were located in the same room with constant environmental conditions such as temperature (19-24°C) and humidity (50-60%). They all had free access to water and were fed and weighed daily between the hours of nine and eleven am every morning throughout the duration of the study. The animals were allowed to acclimatize in the environment for one week before the commencement of the study (carbohydrate administration), which lasted for two weeks

2.2. Experimental Design

The diet composition differing only in local carbohydrate (CHO) sources were given to the experimental and control animals. The group A animals received 73.75g of garri (processed and fried cassava grains) in a 100g diet, while group B animals received similar quantity of rice flour (pre-boiled) in 100g diet. Group C however received 73.75g of yam flour (pre-boiled)whereas group D (control) received the same quantity of sucrose in 100g diet. The rest of the 100g diet were comprised of Casein (vitamin free) 15.85, vegetable oil (4g), salt mixture (4g), vitamin mixture (2.2g) and L-methionine 0.2g (Table 1).

whereas group D (control) received the same quantity of sucrose in 100g diet. The rest of the 100g diet were comprised of Casein (vitamin free) 15.85, vegetable oil (4g), salt mixture (4g), vitamin mixture (2.2g) and L-methionine 0.2g (Table 1).

2.3.Sample Collection and Processing

At the end of the feeding period, blood was collected from the jugular vein. About two (2) millilitres each was transferred into fluoride and EDTA anticoagulant bottles for blood sugar and plasma tryptophan (TRP) determinations respectively.

Table 1. Diet Compositions (g) given to the Different Groups of the Test and Control Animals.

Group A (Garri)	Weight Per 100g	Group B (Rice)	Weight Per 100g	Group C (Yam)	Weight Per 100g	Group D (Sucrose)	Weight Per 100g
Garri (fried)	73.75 g	Rice floor (pre-boiled)	73.75 g	Yam floor (pre-boiled)	73.75 g	Sucrose	73.75 g
Casein (vitamin free)	15.85 g	Casein (vitamin free)	15.85 g	Casein (vitamin free)	15.85 g	Casein (vitamin free)	15.85 g
Vegetable oil	4.0 g	Vegetable oil	4.0 g	Vegetable oil	4.0 g	Vegetable oil	4.0 g
Salt mixture	4.0 g	Salt mixture	4.0 g	Salt mixture	4.0 g	Salt mixture	4.0 g
Vitamin mixture	2.2 g	Vitamin mixture	2.2 g	Vitamin mixture	2.2 g	Vitamin mixture	2.2 g
L-methionine	0.2 g	L-methionine	0.2 g	L-methionine	0.2 g	L-methionine	0.2 g

The rats were sacrificed by decapitation immediately after blood collection at approximately the same time of the day (ten to eleven am) to eliminate diurnal variations, especially with respect to serotonin (5-HT) level in the brain. The whole brain was removed as quickly as possible from the skull leaving out the olfactory lobe and making sure that the medulla oblongata was not excluded. The brain was removed into a chilled weighed plastic bottle containing known volume (about ten millilitres) of 0.3 M sucrose solution. The bottle and its content were re-weighed and the difference gave the weight of the whole brain. When the brain sample was not immediately processed, it was quickly transferred to a deep freezer (-40°C) or after it has been frozen using liquid nitrogen.

2.4.Analytic Methods

Blood sugar estimation was done using copper reduction method of Hallswood and Strookman [21] whereas the protein content of the brain homogenate was determined using a slight modification of the method of Lowry et al., [22], with bovine serum albumin (BSA) as standard the optical densities were read using spectronic-20 (Bansch and Comb) spectrophotometer and the concentrations extrapolated from a standard curve. The plasma and brain TRP were determined using the fluorimetric method of determination of plasma and brain TRP on aliquots containing 5-HT [23], which was essentially that of Denkla and Dewey [24]. The modification of Bloxam and Warren [25] was also used. The determination of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) was based on the fluorimetric method, slightly modified by Curzon and Green [26]. Both assays were performed with Ratio-fluorometer-2 (Rand optical Company Inc, USA). The concentrations were read-off from standard curves.

A 10% brain homogenate was made in 0.32M sucrose solution containing 1mM 2-mercapto-ethanol after which extraction of TRP, 5-HT and 5-HIAA from the brain homogenate was as described by Knott and Curzon [23] with modifications. Exactly 0.6 millilitres of the brain homogenate was added into a tube containing 3.0 millilitres of ice-cold acidified n-butanol and mixed with vortex mixer at 3000rpm for five minutes at 4°C. Then 2.5 millilitres of the supernatant (n-butanol phase) was transferred into fifteen millilitres stoppered tube containing five millilitres of ice-cold n-heptane and mechanically agitated with 1 millilitre of freshly prepared 0.1% acid cystein for ten minutes. The tube was centrifuged and the bottom (aqueous) layer separated for TRP and 5-HT assay. The organic phase (mixture of n-butanol and n-heptane phase) was further extracted into freshly 0.1% buffered cysteine by mechanical mixing in a fifteen millilitres stoppered glass centrifuge tube as above with five millilitres of the organic phase for ten minutes. The bottom (aqueous) layer was separated for 5-HIAA assays after centrifugation of the tube, as above.

2.5. Statistical Analysis

The data obtained were analyzed by students' T-test at 95% and 99% confidence limits and 3 degrees of freedom, using the statistical package for social sciences (SPSS) software. Results are presented as mean ± SEM, with P values of <0.05, <0.01 and <0.001 considered significant.

Table 2. Effect (Mean ± SEM) of different local carbohydrate diets (garri, yam and rice) on Body and Brain weights, Blood sugar, Plasma and Brain tryptophan, Brain protein, 5-HT and 5-HIAA in Weanling male albino Wistar rats after two weeks on the diet.

	Group A (Garri fried)	Group B (Pre-boiled rice)	Group C (Pre-boiled Yam)	Group D (Sucrose diet)
Body weight (g)	102 ± 6.35	109.04 ± 7.17	102 ± 6.55	109.62 ± 9.15
Body weight (g)	1.41 ± 0.03	1.54 ± 0.06	1.43 ± 0.06	1.61 ± 0.07
Brain weight Bodyweight ratio (%)	1.38 ± 0.47	1.41 ± 0.83	1.40 ± 0.91	1.47 ± 0.77
Blood sugar (mg/100ml)	64.4 ± 1.25	72 ± 4.15	71.8 ± 1.28	83.4 ± 2.30
Plasma Tryptophan (µg/ml)	16 ± 0.26	21 ± 0.57	28 ± 0.72	33 ± 0.72
Whole brain protein (µg/g)	124.75 ± 10	124.00 ± 8.75	125.00 ± 10	126.00 ± 32.5
Brain TRP (µg/g)	16.56 ± 0.78	20.88 ± 3.57	21.60 ± 4.2	23.76 ± 3.4
Brain 5-HT(µg/g)	0.322 ± 0.09	0.526 ±0.04	0.652 ± 0.04	0.609 ± 0.0
Brain 5-HIAA (µg/g)	0.342 ± 0.07	0.346 ± 0.06	0.428 ± 0.04	0.583 ± 0.15

3.Results

The effects on brain TRP level, as presented in table 3, shows that sucrose diet increased brain TRP levels significantly over the groups fed on garri, yam and rice (P<0.001; <0.05; <0.05) respectively. However, the local carbohydrate sources, when compared with one another, showed no significant difference. The table also showed that the type of local CHO consumed had no significant effect on the brain protein concentration of the animals, compared with the control. Serotonin (5-HT) level in the control group was however, significantly higher (P<0.001; <0.001; <0.05) than the other three groups fed with garri, rice and yam respectively (table 3). Also the brain 5-HT concentrations of animals fed on rice and yam were significantly higher (P<0.05; P<0.001) than those fed with garri. No significant difference (P>0.05) was observed between the groups fed with yam and rice. The brain 5-hydroxyindoleacetic acid (5-HIAA) concentration in the control animals was significantly higher (P<0.01) than animals fed on garri and rice and (P<0.05) for those fed on yam.

Table 3. The effects on brain TRP level

	Group A (Garri fried)	Group B (Pre-boiled rice)	Group C (Pre-boiled Yam)
Body weight (g)	P<0.05	P>0.05	P<0.05
Brain weight (g)	P<0.001	P<0.001	P<0.001
Brain weight Bodyweight ratio (%)	P>0.05	P>0.05	P>0.05
Blood sugar (mg/100ml)	P<0.001	P<0.001	P<0.001
Plasma Tryptophan (μg/ml)	P<0.001	P<0.001	P<0.05
Whole brain protein (μg/g)	P>0.05	P>0.05	P>0.05
Brain TRP (μg/g)	P<0.001	P<0.05	P<0.05
Brain 5-HT(μg/g)	P<0.001	P<0.001	P<0.05
Brain 5-HIAA (μg/g)	P<0.01	P<0.01	P<0.05

4.Discussion

The study tested the hypothesis that local carbohydrate sources might influence the metabolism of serotonin in wistar rats and the results reveals that various local carbohydrate (CHO) sources, when given in equal amounts produce different blood glucose concentrations and responses. This implies that individuals requiring CHO restrictions for reasons of lowering blood glucose should avoid yam and rice, or take them in very minimal quantities whereas garri may be given liberally. The possible mechanisms for different glucose responses for the three local CHO sources under investigation may be related to the differences in digestion and absorption. More complex CHO are usually being digested more slowly and therefore gives lower glucose response [6]. This may explain why sucrose, which is a disaccharide, produced a comparatively higher sugar concentration in this study within the period of the experiment. However, food rich in fibre content like garri used in this study induced lower blood glucose responses. Other factors to be considered include the method of preparation of food; gastric emptying time which may differ for different CHO; gastrointestinal insulinogenic hormones secretion and stimulation and lastly the rate of removal of glucose from plasma by insulin secreted with different stimulations as a result of different CHO sources consumed. Whatever the mechanism, it may not suffice to restrict CHO dietary intake in certain disorders in order to lower blood glucose concentration, rather, a more specific therapeutic recommendation should be made by specifying the type of CHO to be used in order to achieve a particular goal. The influence of different CHO sources on plasma and brain tryptophan (TRP) concentrations could also be as a consequence of the rate of insulin response to the CHO sources. Lyons and Truswell [16] found that high glucose levels increased the rate of synthesis of proinsulin and insulin approximately eight folds, whereas Fernstrom and Wurtman [1] showed injection of the hormone insulin into fasting rats rapidly elevates serum and brain TRP concentrations of the large neutral amino acids (LNAAs) as they are moved into the tissues, hence the plasma concentration of TRP is consequently increased (sparing effect of TRP). Fernstrom et al [6] also showed that ingestion of a single CHO meal stimulated insulin which increased the uptake of TRP and consequently increased synthesis of serotonin (5-HT). The basis for the predictable diet-related changes in brain TRP level, resulting from high glucose concentration of rats fed on sucrose compared to the other CHO sources arise from the carrier mechanism for TRP transport into the brain [16]. The significant increase in plasma and brain TRP following the consumption of sucrose compared to other main local CHO dietary sources (yam, rice and garri), could also be due to the additional amino acids and proteins contained in these CHO sources [10]. This could lead to increased serum concentrations of the competing neutral amino acids, giving rise to proportionate "TRP-sparing effect". Wurtman et al., [20] has shown that the TRP content per hundred grams of pre-boiled rice and dried yam are the same. However, rice, in addition, contains competing amino acids in higher concentrations than yam. This may therefore explain why the level of brain TRP concentrations in animals fed yam in this study was higher than those fed rice, despite the plasma level of TRP and glucose response of rice compared to yam. This study also shows that, generally, there were increased plasma and brain TRP levels and hence, 5-HT levels and a decrease in 5-hydroxyindole acetic acid (5-HIAA), mainly due to these CHO sources. The increased brain 5-HT following the rise in brain TRP is expected since a metabolic relationship exists between TRP, 5-HT and 5-HIAA. Hence any factor that influences either the level of plasma TRP or its uptake into the brain will subsequently affect the synthesis of the neurotransmitter, 5-HT. Consequently, the insulinogenic action of these CHO sources and their effect on plasma and brain TRP further illustrates the respective levels of brain 5-HT obtained. The significant decrease in brain 5-HT level resulting from ingestion of garri as a CHO source could be attributed in part to the effect of cyanide from cassava, which has been shown to be present in little quantity in the processed garri, usually in the form of linamarin. Cyanide is a well-known enzyme inhibitor and may most likely inhibit the action of the enzyme TRP hydroxylase which is the rate-limiting enzyme in the synthesis of 5-HT. Another factor which may have, in addition enhanced positively the effect of yam and rice (apart from the insulinogenic action and glucose response) in the synthesis of the neurotransmitter 5-HT, could be the presence of additional vitamins and hence co-factors, which could enhance the activity of the enzymes involved in the metabolism of 5-HT. The level of 5-HIAA, which is the major breakdown product of 5-HT, is a reflection of the amount of 5-HT synthesized by the brain in these rats. A significant difference was observed in the body and brain weights of animals fed on sucrose and rice as CHO sources compared with those fed on yam and garri respectively. These differences could be attributed to factors such as caloric content of the CHO, rate of absorption into the tissues (due to their fibre content), glucose response and insulinogenic effect and subsequently, the rate of movement of amino acids and other substrates into the muscle and brain tissues for anabolic purposes.

5. Conclusion

Sucrose gave the highest concentration of both blood and brain TRP, followed by yam and then rice. Garri gave the lowest value, possibly due to its high fiber content, decreased glucose response and insulinogenic action, followed by reduced plasma and brain TRP and consequently brain 5-HT synthesis. There is also possible interference of enzyme inhibitors, like cyanide. Thus, garri seems to be the CHO of choice in patients requiring CHO restrictions to lower blood glucose. Also sucrose should be given to patients with psychiatric disorders resulting from diminished.

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