Changes in Serum Amino Acid Content and Dopamine- β -Hydroxylase Activity and Brain Neurotransmitter Interaction in Cats Fed Casein with or without Gluten or Gliadin

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(Received October 26, 1987)

Summary Adult cats adapted to a semi-purified casein diet were used to study the effects of chronic doses of gluten or gliadin administered *per os* for 16 consecutive days, for a total of 700 mg per kg per day, on various blood and brain parameters. The *ad libitum* food intake, serum amino acid content, brain, adrenal, and serum dopamine- β -hydroxylase activity, and cerebral GABAergic, cholinergic, dopaminergic, noradrenergic, and serotonergic metabolism of cats fed casein gluten or gliadin were studied. In the brain, the wheat proteins favored mesolimbic and nigrostriatal dopaminergic pathways and decreased the choline acetyltransferase and glutamic acid decarboxylase activities in these pathways thus suggesting an interaction between the corresponding neurotransmitters. The decreased food intake in cats fed gluten or gliadin was paralleled by an increased noradrenaline, dopamine, and serotonin contents in the brainstem areas. In treated cats the adrenal and serum dopamine- β -hydroxylase activities were decreased whereas this enzyme activity in the brain remained normal. The ratio of serum total tryptophan or tyrosine to the sum of six neutral amino acids were significantly decreased and inversely correlated with serotonin and catecholamine contents in the brain. Biochemical peripheral and cerebral disturbances observed following chronic gluten or gliadin administration

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could provide some basis for correlated behavioral properties of wheat peptides.

Key Words: gluten-gliadin, neurotransmitters, amino acids, enzymes, cat

Early studies have described dietary proteins, namely, gluten and its major component gliadin, as pathogenic factors involved in coeliac disease [1] as well as in psychiatric disorders in man [2]. In commenting on the similarities between symptoms of schizophrenia and those that occur in coeliac disease, Dohan and Grasberger [3] and Dohan *et al.* [4] reported that schizophrenic patients on a cereal-free and milk-free diet improved at a significantly faster rate than other patients. An uncontrolled trial in 16 cases of schizophrenia revealed an improvement of the symptoms in two of them on a low gluten diet [5]. On the other hand, no behavioral changes were observed after gluten challenge in chronic cases of schizophrenia who remained on a cereal-free and milk-free diet, according to the study of Potkin *et al.* [6].

It has been suggested that wheat proteins have an exacerbating effect on schizophrenic patients by diminishing the response to neuroleptics [7], whereas Osborne *et al.* [8] did not find any evidence that gluten interferes with the absorption of neuroleptic medication. An improvement of behavioral status was somewhat demonstrated in adult coeliac patients fed a gluten-free diet [9].

Wheat proteins were found to exert their biological activity through large molecules of gluten derivatives that can reach the brain in schizophrenia symptom-producing amounts [10, 11]. Exorphins (exogenous peptides derived from gluten) were suggested to be responsible for the relationship between wheat gluten and schizophrenia [11, 12].

The purposes of the present work were to use two types of wheat proteins, gluten and gliadin, administered chronically to normal cats in order to compare the dietary effects on 1) the body weight and food and water intakes; 2) the serum dopamine- β -hydroxylase (DBH) activity and neutral amino acids concentrations; 3) the adrenal DBH activity, 4) the brain choline acetyltransferase (ChAT), glutamic acid decarboxylase (GAD), and DBH activities, and the serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA) and dopamine (DA) content in 10 brain areas, and to analyse the data in the light of the biochemical mechanisms associated with schizophrenia.

MATERIALS AND METHODS

Animals. Twenty-four adult mongrel cats of both sexes, initially weighing 2.94 ± 0.08 kg, were adapted to environmental conditions for at least three weeks and were judged healthy by a veterinarian. They were housed in individual stainless steel cages in a room controlled at constant temperature (21°C) and humidity

(55%) with fluorescent lights and background music from 06:00 to 18:00 h. The experiment was conducted during the spring.

Biochemical procedures. Cats were guillotined without anesthesia between 08:00 and 11:30 h. Adrenals, liver, and brain were rapidly set aside and the following brain structures dissected out: the neostriatum, thalamus, hypothalamus, piriform lobe, hippocampus, the ventral and dorsal parts of the mesencephalon (Mes), and the respective raphe nuclei of the Mes, pons and medulla. Blood was collected and centrifuged for 15 min at 2,500 rpm in a refrigerated (4°C) Sorvall RC-5B to separate the serum, which was then stored at -60°C. 5-HT, 5-HIAA, NA, and DA were isolated on Sephadex G-10 as described by Early and Leonard [13]. 5-HT and 5-HIAA were determined according to Curzon and Green [14]. NA was oxidized using the technique of Maickel *et al.* [15], whereas DA was determined according to the method of Welch and Welch [16]. Recoveries of 5-HT, 5-HIAA, NA, and DA were $90 \pm 3\%$, $83 \pm 4\%$, $91 \pm 4\%$, and $87 \pm 4\%$, respectively. All estimates were corrected for losses.

Enzyme measurement. Brain ChAT activity was measured according to the method described by Dontigny [17]. Results are expressed in nmol of acetylcholine formed $\cdot h^{-1} \cdot mg$ protein $^{-1}$. Brain, adrenal, and serum DBH activities were determined by the method of Kato *et al.* [18] as modified by Roberge and Charbonneau [19]. Results are expressed in nmol of octopamine formed $\cdot h^{-1} \cdot g$ protein $^{-1}$, or per total wet weight of adrenals or ml of serum.

Brain GAD activity was measured according to a radiochemical method described by Kravitz *et al.* [20]. Results are expressed in nmol of γ -aminobutyric acid (GABA) formed $\cdot h^{-1} \cdot mg$ of protein $^{-1}$.

Protein determination. Protein concentrations were measured by the method of Bradford [21] using bovine serum albumin as standard.

Amino acid determination. Four hundred microliter of serum and 400 μl of dry 5% sulfosalicylic acid were stirred together for 5 min, refrigerated 1 h at 4°C and then centrifuged for 15 min in a Sorvall centrifuge (RC-5B) at 2,500 rpm. Two hundred microliter of the supernatant diluted 1 : 1 with Li-S buffer and filtered through a Millipore filter (0.22 μm) was used for the analysis. The amino acid analysis was performed with a Beckman amino acid analyzer (Model 6300, Beckman Instruments, Palo Alto, CA) to measure the following amino acids: arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, tyrosine, phenylalanine, valine, threonine, tryptophan, glutamic acid, and methionine. The instrument was calibrated with a standard mixture of 22 amino acids (2.5 $\mu mol/ml$), including tryptophan and ornithine. Results are expressed in $\mu mol/100$ ml of serum. The same method was used to measure the amino acid content of casein, gluten, and gliadin.

Diet and wheat proteins administration. The semi-purified casein diet was formulated to meet nutrient requirements of cats as specified by the National Academy of Sciences, Canada [22]. The requirements were 28% for protein and 9% for fat including two essential fatty acids (linoleic and arachidonic acids); and the caloric needs were 70 kcal per kg of body weight for inactive adult cats. To

meet the nutritional requirements for cats, the daily dietary ration (dry weight) was 50 g. In diets, the source of fat was corn oil to fulfill the requirements for linoleic and arachidonic acids. Table 1 shows the percentage and the quantity (g, dry weight) of nutrients in the semi-purified diet. Food and tap water were provided *ad libitum*.

Six cats were used as controls, and 11 and 7 cats received 700 mg per kg per day of gluten and gliadin, respectively. Both gluten and gliadin were administered orally in gelatin capsules while controls received empty capsules at the same time. The daily dose was fractioned and administered at 4-h intervals for 16 consecutive days. On the 17th day, cats received the last dose at 10-min intervals and were sacrificed 1 h later with control cats interspersed between treated animals.

Statistical analysis. Mean, standard deviation, standard error of the mean (SEM), and Student's *t*-test were calculated according to Snedecor and Cochran [23].

Chemicals. 5-HT (creatinine sulfate complex), 5-HIAA (dicyclohexylammonium salt), NA (DL-arterenol HCl), DA (3-hydroxytyramine HCl), tyramine,

Table 1. Composition of diets (dry weight).^a

Casein diet (%)			
Protein			30.7
Corn oil			10.0
Corn starch			23.9
Sucrose			19.2
Alphacel			5.0
Salt mix			10.0
Vitamin mix			1.1
CHO: protein ratio ^b			1.4
Energy density			3.9
Amino acids ^c	Casein	Gluten (g per 16 g N)	Gliadin
Arginine	3.45	3.09	2.50
Cystine	1.97	2.15	1.63
Glycine	1.89	3.49	2.07
Histidine	3.02	2.08	2.43
Isoleucine	3.58	2.13	4.17
Leucine	9.07	6.57	7.08
Tyrosine	5.07	3.02	3.13
Phenylalanine	4.54	5.79	5.55
Valine	4.57	2.71	3.87
Methionine	1.91	1.32	0.71
Lysine	7.43	1.57	0.63
Threonine	3.97	2.44	1.72
Tryptophan	0.93	0.47	0.68
Glutamic acid	22.30	38.20	41.00

^a Prepared in our laboratory with purified casein (99% protein). ^b Total carbohydrates (CHO) to protein ratio. ^c Amino acids from gluten, gliadin, and casein were determined by the method described in the Materials and Methods section.

sodium-m-periodate, fumaric acid, pargyline HCl, ascorbic acid, octopamine, sodium-m-periodate, catalase, Sephadex G-10 and Dowex 50W-4X were purchased from Sigma Chemical Company (St. Louis, MO). Trichloroacetic acid, sodium bisulfite, and monobasic and dibasic sodium phosphate were obtained from Fisher Scientific (Ste-Foy, Québec). Sodium acetate and N-ethyl-maleimide were purchased from BDH (Ville St. Laurent, Québec). Columns (glass-barrel ECONO-columns, i.d. 7×40 mm) were obtained from Bio-Rad Laboratories Ltd. (Richmond, CA). Empty gelatin capsules (No. 0) were from Parke, Davis Ltd. (Brockville, Ontario). Casein, salt mix, vitamin mix and alphacel were supplied by ICN Nutritional Biochemicals (Montréal, Québec). Wheat gluten (WHETPRO-80) was from Industrial Grain Products Ltd. (Montréal, Québec) and gliadin (crude from wheat gluten) from Sigma Chemical Company, (St. Louis, MO). Bovine serum albumin was purchased at ICN Pharmaceuticals Inc. (Cleveland, OH). Li-S buffer and the 22 amino acids used as standard were purchased from Beckman Company (Palo Alto, CA). [^{14}C]Choline, [^{14}C]acetylcoenzyme A, [^{14}C]glutamic acid, and [^{14}C]- γ -aminobutyric acid were purchased from New England Nuclear, Canada.

RESULTS

Since no significant difference was found between results obtained in cats fed casein diet with gluten or gliadin, most results obtained from these treated groups were pooled. The initial body weight of control and treated cats was not significantly different from that at the end of the 16-day period. On the other hand, the adrenal weight was significantly higher in cats receiving gluten or gliadin than in control cats (controls, 231 ± 19 mg; treated, 273 ± 10 mg; $p < 0.05$). The mean daily food (dry weight) and water intake were significantly higher in controls than in cats receiving gluten or gliadin (food: controls, 32 ± 1 g; treated, 23 ± 1 g; $p < 0.01$; water: control, 73 ± 2 ml; treated, 61 ± 2 ml; $p < 0.01$).

Data presented in Table 2 show the dietary intake and the serum concentration of neutral amino acids in control and gluten- and gliadin-treated cats. Cats fed the casein diet with gluten or gliadin maintained their serum neutral amino acid content comparable to controls, except for isoleucine and methionine, which were significantly increased ($p < 0.05$), and for total tryptophan, which was decreased ($p < 0.05$) (Table 2). The total amount of the seven neutral amino acids was slightly higher in the serum of cats fed the casein diet with gluten or gliadin than in the serum of cats fed the casein diet alone. The ratio of total tryptophan to the sum of the six neutral amino acids (6 NAA) was significantly lower ($p < 0.01$) in cats fed casein diet with gluten or gliadin than in control cats fed casein, as was the ratio of tyrosine to 6 NAA ratio ($p < 0.05$) (Table 2).

In Table 3, the effects of the wheat proteins, gluten and gliadin, on 5-HT and 5-HIAA concentrations in 10 brain areas are presented. In the gluten and gliadin groups, the 5-HT content was increased in the thalamus ($p < 0.05$), hypothalamus ($p < 0.05$), both dorsal and ventral Mes ($p < 0.05$) and medulla raphe nuclei

Table 2. Daily dietary intake of neutral amino acids (NAA) and their concentrations in serum of cats fed casein with or without gluten or gliadin.

Amino acids	Dietary intake ^a (mg/day)		Serum concentration ($\bar{X} \pm \text{SEM}$) ($\mu\text{mol}/100 \text{ ml}$)	
	Casein (6)	Casein plus gluten or gliadin (13)	Casein (6)	Casein plus gluten or gliadin (13)
Valine	277	341	33.5 $\pm$ 2.6	36.2 $\pm$ 3.3
Isoleucine	217	280	13.0 $\pm$ 0.8	16.4 $\pm$ 1.0*
Leucine	550	681	24.1 $\pm$ 2.2	26.1 $\pm$ 2.2
Tyrosine	307	366	9.8 $\pm$ 0.7	10.2 $\pm$ 0.6
Phenylalanine	275	383	12.1 $\pm$ 1.0	12.5 $\pm$ 0.5
Methionine	116	135	5.9 $\pm$ 0.4	7.7 $\pm$ 0.5*
Total tryptophan	56	68	6.0 $\pm$ 0.6	4.0 $\pm$ 0.4*
Total tryptophan/6 NAA ratio ^b	0.032	0.031	0.059 $\pm$ 0.003	0.038 $\pm$ 0.004**
Tyrosine/6 NAA ratio ^c	0.206	0.194	0.116 $\pm$ 0.003	0.096 $\pm$ 0.006*

^a The dietary intake was calculated from the diet amino acids (Table 1) and the daily food intake. Figures in parentheses indicate the number of cats. ^b Valine, isoleucine, leucine, tyrosine, phenylalanine, methionine. ^c Valine, isoleucine, leucine, phenylalanine, methionine, total tryptophan. * $p < 0.05$; ** $p < 0.01$.

Table 3. Brain 5-HT and 5-HIAA content in cats fed casein with (treated cats) or without (controls) gluten or gliadin.^a

Structures	5-HT		5-HIAA	
	Controls (6)	Treated cats (17)	Controls (6)	Treated cats (17)
Neostriatum	1.39 $\pm$ 0.11	1.54 $\pm$ 0.12	1.04 $\pm$ 0.07	1.02 $\pm$ 0.05
Thalamus	0.90 $\pm$ 0.05	1.19 $\pm$ 0.07*	1.27 $\pm$ 0.08	1.32 $\pm$ 0.04
Hypothalamus	2.44 $\pm$ 0.23	2.98 $\pm$ 0.11*	1.08 $\pm$ 0.11	1.37 $\pm$ 0.06*
Hippocampus	1.01 $\pm$ 0.03	1.04 $\pm$ 0.04	0.96 $\pm$ 0.08	0.97 $\pm$ 0.03
Piriform lobe	0.98 $\pm$ 0.11	0.97 $\pm$ 0.05	1.29 $\pm$ 0.11	1.17 $\pm$ 0.06
Dorsal Mes	1.86 $\pm$ 0.13	2.32 $\pm$ 0.12*	1.61 $\pm$ 0.10	1.87 $\pm$ 0.11
Ventral Mes	0.78 $\pm$ 0.05	0.97 $\pm$ 0.05*	0.40 $\pm$ 0.04	0.40 $\pm$ 0.03
Raphe nuclei (Mes)	2.79 $\pm$ 0.12	2.77 $\pm$ 0.14	2.29 $\pm$ 0.22	1.81 $\pm$ 0.09*
Raphe nuclei (Pons)	1.35 $\pm$ 0.10	1.18 $\pm$ 0.07	1.80 $\pm$ 0.17	1.50 $\pm$ 0.09
Raphe nuclei (Medulla)	1.46 $\pm$ 0.12	1.85 $\pm$ 0.10*	1.36 $\pm$ 0.12	1.07 $\pm$ 0.05*

^a Results are expressed in μg of 5-HT or 5-HIAA per g tissue ($\bar{X} \pm \text{SEM}$). Figures in parentheses indicate the number of animals used. * $p < 0.05$.

($p < 0.05$). A significant rise in 5-HIAA level was found in the hypothalamus ($p < 0.05$), whereas the 5-HIAA content was decreased in both the Mes and medulla raphe nuclei ($p < 0.05$).

In cats fed gluten or gliadin, the NA content was decreased in the neostriatum ($p < 0.01$), thalamus ($p < 0.05$), and piriform lobe ($p < 0.05$), but increased in the

Table 4. Brain NA and DA content in cats fed casein with or without gluten or gliadin.^a

Structures	NA		DA	
	Controls (6)	Treated cats (16)	Controls (6)	Treated cats (16)
Neostriatum	0.30±0.02	0.24±0.01**	12.44±0.94	12.20±0.40
Thalamus	0.33±0.03	0.25±0.02*	0.20±0.03	0.20±0.01
Hypothalamus	1.87±0.22	1.84±0.14	0.18±0.01	0.23±0.01**
Hippocampus	0.20±0.01	0.18±0.01	0.21±0.04	0.17±0.01
Piriform lobe	0.52±0.02	0.40±0.03*	0.41±0.02	0.43±0.03
Dorsal Mes	0.42±0.04	0.58±0.04*	0.36±0.04	0.41±0.02
Ventral Mes	0.25±0.02	0.21±0.02	0.63±0.01	0.72±0.02*
Raphe nuclei (Mes)	0.54±0.08	0.58±0.03	0.21±0.03	0.34±0.03*
Raphe nuclei (Pons)	0.68±0.06	0.87±0.05*	0.28±0.04	0.32±0.03
Raphe nuclei (Medulla)	0.28±0.05	0.72±0.06***	0.29±0.02	0.26±0.02

^a Results are expressed in μg of NA or DA per g of tissue ($\bar{X}\pm\text{SEM}$). Figures in parentheses indicate the number of animals used. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 5. Brain choline acetyltransferase (ChAT) activity and adrenal, serum and brain dopamine- β -hydroxylase (DBH) activity in cat fed casein with or without gluten or gliadin.

Structures	ChAT ^a		
	Controls	Gluten	Gliadin
Neostriatum	50±4	47±5	30±5*
Piriform lobe	49±10	44±8	39±4
Thalamus	6.2±0.3	5.4±0.7	4.7±0.4*
Ventral Mes	10.7±0.8	6.7±0.8**	7.0±0.7**

Structures	DBH ^b	
	Controls	Treated cats
Adrenal	81.1±4.6 (6)	66.7±3.8* (12)
Serum	18.5±1.7 (8)	12.0±1.5** (12)
Hypothalamus	5.5±0.16 (6)	5.3±0.40 (12)
Thalamus	5.1±0.22 (6)	4.7±0.29 (17)
Raphe nuclei (Mes)	3.3±0.27 (6)	3.5±0.19 (12)

^a Results are expressed in nmol of acetylcholine formed·h⁻¹·mg of protein⁻¹ ($\bar{X}\pm\text{SEM}$).

The number of cats is 6. ^b Results are expressed in nmol of octopamine formed·total weight (adrenal)⁻¹·h⁻¹·ml of serum⁻¹·h⁻¹· μg of protein (brain tissues)⁻¹·h⁻¹ ($\bar{X}\pm\text{SEM}$). Figures in parentheses indicate the number of cats. * $p<0.05$; ** $p<0.01$.

dorsal Mes ($p<0.05$) and in the pons and medulla raphe nuclei ($p<0.05$ and $p<0.001$, respectively), when the results were compared with the respective control values (Table 4). The wheat proteins produced an increased DA content in the hypothalamus ($p<0.01$), ventral Mes ($p<0.05$), and Mes raphe nuclei ($p<0.05$).

Table 5 presents the variations in DBH activity in the adrenal, serum, hypothalamus, thalamus, and raphe nuclei in control and treated cats and the effects of gluten and gliadin on ChAT activity in different brain areas. In the adrenal

($p < 0.05$) and serum ($p < 0.01$) of treated cats, DBH activity was significantly decreased, whereas no significant change in DBH activity was observed in the brain structures examined. A significant decrease in ChAT activity was observed in the neostriatum ($p < 0.01$) and thalamus ($p < 0.05$) in cats fed the casein diet with gliadin but not in cats fed that with gluten. However, a significant decrease in ChAT activity was observed in the ventral Mes in both groups of cats ($p < 0.01$).

GAD activity measured in the ventral Mes was significantly decreased in cats fed the casein diet with gliadin (4.25 ± 0.32 nmol of GABA formed $\cdot h^{-1} \cdot mg$ protein $^{-1}$ $p < 0.05$) when compared with the activity in cats fed the casein diet containing gluten or the casein diet alone (5.6 ± 0.37 and 5.6 ± 0.42 nmol of GABA formed $\cdot h^{-1} \cdot mg$ protein $^{-1}$, respectively).

DISCUSSION

The present study has demonstrated that wheat proteins, gluten or gliadin, given for 16 consecutive days 1) diminish daily food and water intakes; 2) affect differently monoaminergic systems in cat brain, dissociating the brainstem areas from the diencephalic areas; 3) decrease the brain GAD and ChAT activities, leaving unaffected the brain DBH activity; 4) decrease the adrenal and serum DBH activity; and 5) increase the serum isoleucine and methionine concentrations but decrease the serum total tryptophan content.

A significantly increased 5-HT, NA, and DA content in the brainstem areas was observed in cats fed the casein diet with gluten or gliadin for 16 consecutive days. The high content of biogenic amines in the brainstem areas could be associated with a reduced turnover rate or at least a lower release of these amines, as reported by Hery *et al.* [24] and as indicated by the significantly increased ratio of 5-HT to 5-HIAA observed in the medulla raphe nuclei in treated cats (controls, 1.19 ± 0.13 ; treated, 1.67 ± 0.11 ; $p < 0.05$). Moreover, to this increased biogenic amine content corresponds a decrease in ChAT and GAD activities in the brainstem areas, demonstrating a biochemical balance between the biogenic amine group (5-HT, DA, and NA) and the neurotransmitters, acetylcholine and GABA.

It is obvious to pinpoint that among the biogenic amines involved in the functioning of the brainstem reticular mechanism, NA and 5-HT are the most frequently associated with either arousal [25, 26] or hunger and satiety [27] or the state of chronic hyperarousal observed in schizophrenic patients [28]. In the present study, significant 5-HT and NA increases were found in the medulla raphe nuclei of treated cats, which brain area is involved in the regulation of hunger and satiation mechanisms. In fact, the disturbed NA and 5-HT content in the medulla raphe nuclei might be related to the simultaneous decrease in food and water intakes observed in cats fed the casein diet with gluten or gliadin. Moreover, the present results suggest that gluten and gliadin may act as anorexigenic agents in the same way as amphetamines in man and animals [29–31], potentiating the mesolimbic dopaminergic system in the central nervous system (CNS). In this respect, appetite

suppression in coeliac and schizophrenic patients is often observed in the course of the evolution of these pathologies [32].

The present data have demonstrated that all biogenic amines were increased in cell bodies of the brainstem of treated cats, whereas an inverse relationship was observed between NA and 5-HT content in the diencephalic areas. Indeed, to a significant decrease in the diencephalic NA content corresponded a significant increase in 5-HT level, dissociating the physiological behavior of these amines on a neuroanatomical basis as previously reported by Roberge [33] and Roberge *et al.* [34]. On the other hand, DA and NA have been suggested to be dynamically and functionally interrelated in different behavioral situations and in schizophrenia [35–37]. Numerous biochemical studies on human post-mortem brains from schizophrenics and from patients with related psychotic conditions have shown increased central DA concentrations as well as decreased ChAT and GAD activities [38–40]. In the present study, the low ChAT activity observed in the ventral Mes, neostriatum and thalamus, and the low GAD activity associated with the increased DA content in the ventral Mes of treated cats provide strong similarities with the previous studies done in human post-mortem brains from schizophrenics.

In cats fed the casein diet with gluten or gliadin, a lower DA utilization or release than in controls is suggested by the decreased NA level in the neostriatum as well as by an increased DA : NA ratio in the neostriatum and Mes raphe nuclei (controls, 39.1 ± 4.4 ; treated, 51.3 ± 2.5 ; $p < 0.05$: controls, 0.43 ± 0.04 ; treated, 0.65 ± 0.06 ; $p < 0.05$: respectively), and parallels the increased DA concentration found in the ventral Mes containing the substantia nigra dopaminergic cell bodies that send numerous projections to the caudate nucleus and putamen [41, 42]. These results might be indicative of an enhanced neuronal activity in the nigro-striatal pathway and are consistent with the DA hypothesis of schizophrenia [43].

The present findings demonstrate in cats fed the casein diet with gluten or gliadin (a) decreased content of serum total tryptophan; (b) lowered ratio of serum total tryptophan to 6 NAA; (c) increased content of brain 5-HT and DA; (d) decreased ratio of serum tyrosine to 6 NAA, and (e) increased or decreased content of brain NA. According to these data, a low ratio of serum total tryptophan to 6 NAA corresponds to a high 5-HT content in the brainstem and diencephalic areas, whereas a low ratio of serum tyrosine to 6 NAA corresponds to a high DA content in brainstem and diencephalic areas but to a high NA content in the brainstem areas and to a low NA level in diencephalic areas. The present data tend to demonstrate that under chronic dietary conditions, serum neutral amino acid ratios do not seem to govern the overall CNS biogenic amine level and the differentiated neuroanatomical pathways present in the diencephalon and brainstem areas have to be taken into consideration. This assumption is in agreement with several studies [34, 44–46] that have attempted to define the interactions between the neurotransmitters on a neuroanatomical basis.

The present work also suggests that the nature, quality, and the digestibility

of dietary protein affect the dietary intake and that the bioavailability of amino acids in the blood may be related to the composition of essential amino acids in the dietary protein [47]. In fact, in cats fed the casein diet with gluten or gliadin, the wheat proteins could have contributed to the decreased dietary intake because of a lower digestibility of these wheat proteins than of casein itself (net protein utilization: 54.1 and 83.5 for gluten and casein, respectively) [48].

Moreover, some clinical biochemical parameters were also disturbed by the dietary protein treatment, such as the lowered adrenal and serum DBH activities. These data are supported by the recent works of Thibault and Roberge [49, 50] and Roberge and Charbonneau [19], who showed that dietary proteins selectively affect the serum DBH activity in cats adapted to different sources of protein.

In summary, the present work shows that feeding chronic doses of gluten or gliadin, peptides derived from wheat proteins and involved in coeliac disease, produces a disturbance in the biogenic amine equilibrium in different neuro-anatomical systems which may provide a partial explanation of some previous studies demonstrating aggravation of schizophrenic symptoms in humans following wheat peptide ingestion as well as behavioral effects related to hunger and satiation mechanisms. In fact, the present data reveal that (1) the quality of the dietary protein source influences the food and water intakes and biochemical blood parameters; (2) the ratio of serum total tryptophan or tyrosine to 6 NAA is not directly correlated with catecholamine and serotonin content in the CNS, and (3) the disturbance in brain biogenic amines induced by chronic ingestion of the wheat peptides, gluten and gliadin, might affect some mechanisms related to hunger and satiation located in the brainstem areas as well as mechanisms underlying some schizophreniform reaction in man, but via brain pathways. It is difficult, however, to conclude that gluten sensitivity is of general relevance in schizophrenia since this disorder is not a homogeneous entity and immunological sensitivity is idiosyncratic [51]. The gluten hypothesis of schizophrenia thus needs to be investigated in very carefully designed studies in which the nutritional approach is considered on the basis of the availability of macro- and micronutrients.

The authors gratefully acknowledge the technical assistance of Ruth Charbonneau and the editorial help of Nicole Seoane, M.Sc. This research was supported by the Natural Sciences and Engineering Research Council of Canada (Studentship to Louise Thibault and grant No. A7946) by CRESAQ and FCAR-équipe, Québec.

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