Effect of PCPA or tryptophan on brain serotonin and on consumption of a high protein or high carbohydrate diet by rainbow trout, *Oncorhynchus mykiss*

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The effect of increased or decreased brain serotonin on consumption of a high protein or a high carbohydrate diet by rainbow trout, Oncorhynchus mykiss, *was evaluated. Groups of rainbow trout were acclimated to either a high protein or a high carbohydrate diet for 6 weeks. Fish then received a dietary pretreatment meal containing 5% p-chlorophenylalanine, which decreased brain 5-HT and 5-HIAA, or 7% crystalline tryptophan, which increased brain 5-HIAA and 5-HIAA/5-HT. Intake of a challenge meal (high protein or high carbohydrate, consistent with the diet to which they had been acclimated) was then determined. Food consumption by fish fed the high protein diet was unaffected by either dietary pretreatment meal. Conversely, both p-chlorophenylalanine and tryptophan pretreatment decreased food consumption by fish fed the high carbohydrate diet. These results suggest that changes in brain serotonin turnover in rainbow trout are not important in controlling intake of a high protein diet. Furthermore, because consumption of the high carbohydrate diet was decreased both by p-chlorophenylalanine, which decreased serotonin turnover, and by tryptophan, which increased serotonin turnover, this also suggests that serotonin turnover may not be an important signal controlling consumption of high carbohydrate diets by rainbow trout.*

Keywords: p-chlorophenylalanine; serotonin; tryptophan; food intake; carbohydrate; protein; rainbow trout, *Oncorhynchus mykiss*

Introduction

The role of brain serotonin (5-HT) in mediating satiety is controversial.[1–5] Many investigators believe it acts to decrease food intake in general.[3,6] As evidence for this hypothesis, these researchers cite experiments demonstrating that intake of all macronutrients is decreased by treatment with drugs that increase 5-HT neurotransmission in the brain. For example, fenfluramine, a drug that stimulates release of 5-HT into the synapse while concomitantly inhibiting re-uptake, decreases intake of carbohydrate (CHO), lipid, and protein.[2,7,8] However, some investigators feel that the role of 5-HT in feeding behavior is macronutrient-specific. By way of example, Leibowitz et al.[9] found that consumption of CHO in the early part of a meal was decreased by injection of 5-HT into the paraventricular nucleus, while intake of lipid and protein was unaffected. Similarly, Li and Anderson[10] provided rats with a choice of two diets that varied in the relative amount of protein and CHO but not in energy content (two-choice, self-selection paradigm), and reported that inhibition of 5-HT synthesis by administration of p-chlorophenylalanine (PCPA), an inhibitor of tryptophan-5-monooxygenase (EC 1.14.16.4), increased the percent of calories selected as CHO. Furthermore, Stal-

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lone and Nicolaidis[11], showed that even when rats pretreated with the 5-HT antagonist, metergoline, were not provided a choice, but rather were fed single diets of fixed macronutrient composition, they still increased intake of the high CHO diet even though this altered overall energy consumption.

Most experiments investigating the effect of pharmacological manipulation of brain 5-HT turnover on subsequent food selection have been conducted in mammals, especially in the omnivorous rat and humans. The rainbow trout, *Oncorhynchus mykiss,* an important aquaculture species, differs from rats and humans not only in being a fish, but also in being a strict carnivore whose natural diet contains very low levels of digestible CHO. Since this fish consumes its prey whole, it does not have the option of selecting from parts of the prey that differ in macronutrient composition (for example, by selective "organ grazing"), but can choose only whether to eat. The role of 5-HT in the control of feeding behavior in trout has not been investigated. However, the distribution of brain 5-HT in trout is very similar to that of mammals,[12–14] and food intake results in changes in brain 5-HT turnover.[15] In addition, provision of inexpensive high CHO diets to trout decreases food consumption and depresses weight gain.[16,17] Because 5-HT is believed to play a role in controlling food (or specifically CHO) intake in mammals, it is important to determine if it plays a physiological role in the carnivorous rainbow trout and, because typical low CHO, fishmeal-based aquaculture diets are expensive, this information may also have economic significance. Therefore, the objective of this experiment was to examine the role of 5-HT in controlling intake of a high CHO diet or of a more natural high protein, low CHO diet by rainbow trout in which 5-HT had been either depleted by PCPA, or enhanced by tryptophan loading.

Materials and methods

Diet preparation and analysis

Two practical fishmeal/soybeanmeal-based diets were formulated as either high protein (HP) or high CHO (HC) modifications of the diet previously described.[15] *(Table 1).* The HP diet was formulated to contain approximately 50% protein (on an "as is" basis), a level recommended for maximal growth of rainbow trout fry[18] and a level that may also be appropriate for broodstock. The HC diet was formulated to contain adequate protein for maximal growth of juvenile trout (35%) and at the same time to contain a level of digestible CHO (35%) that produces both hepatomegaly due to the storage of excess glycogen, and alterations in liver enzyme activity in rainbow trout.[16,19] To maintain the same level of digestible energy it was necessary to vary the quantity of lipid from 16% in the HP diet to 11% in the HC diet. Because Crane and Greenwood[20] observed that the type of dietary fat can alter macronutrient selection by rats, and the use of fishmeal and soybeanmeal as protein sources unavoidably contributes some associated fat to the diet, the relative contribution to the total lipid amount by each dietary lipid source was kept constant in both diets by appropriate inclusion of fish oil and soybean oil. Likewise, because amino acids have a role in feeding behavior,[21] while the quantity of protein varied between the two diets, the amino acid proportions remained constant. The calculated maximal level of digestible CHO in each formulation was 5.7% (HP) and 32.5% (HC) with a calculated protein:CHO ratio of 8.5 (HP) or 1.0 (HC). Diets were processed by steam pelleting in a Laboratory Pellet Mill (California Pellet Mill Co., San Francisco, CA, USA), dried overnight in a forced-air drying oven, and stored at −20° C until required for feeding. Samples of processed diets were analyzed for crude protein by Kjeldahl nitrogen analysis[22] using a Tecator Kjeltec Auto 1030 Analyser (Fisher, Toronto, Ontario, Canada), for lipid by the method of Bligh and Dyer,[23] for moisture by drying at 105° C for 24 hours, and for ash by ignition at 600° C *(Table 1).* Dietary pretreatment meals were formulated by substitution of PCPA (Sigma Chemical Co., St. Louis, MO, USA), tryptophan or alanine (placebo) for wheat middlings and a small amount (3–5%) of the remainder of the diet (excluding the vitamin and mineral premixes); and nitrogen was balanced with alanine *(Table 2).* To distinguish test meal intake from residual stomach contents, dietary pretreatment meals were provided in the form of red (carmine) or green (chromic oxide) diets.* Dietary pretreatment meals were processed by mixing seven parts dry diet with four parts glass-distilled water. The mash was briefly blended and milled into feed-

Table 1 Diet formulation and composition

	HP (high protein)	HC (high CHO)
Ingredients (% inclusion)		
Fishmeal	50	35
Soybean meal	31.4	22
Wheat middlings	7	4.9
Vitamin premix[a]	2	2
Mineral premix[b]	1	1
Fish oil	8.43	4.75
Soybean oil	0.17	—
Glucose	—	30
Cellulose	—	0.35
Analysis[c]		
Protein	54.7	39.3
Lipid	16.4	10.9
Ash	7.7	5.8
Moisture (%)	6.0	8.8

[a]Vitamin premix supplied the following in mg/kg diet (except where units are noted): vitamin A (retinyl palmitate and retinyl acetate), 4165 IU/kg; vitamin D_3, 835 IU/kg; vitamin E (dL-alpha-tocopheryl acetate), 300; vitamin K (menadione sodium bisulphite), 25; thiamin mononitrate, 10; riboflavin, 10; niacin, 99; pyridoxine HC1, 40; D-Ca pantothenate, 100; folic acid 17; biotin 0.5; cyanocobalamin, 0.2; choline chloride, 5500; ascorbic acid, 1000; butylated hydroxytolune, 25; DL-methionine, 3000; alpha floc as carrier.
[b]Mineral premix supplied the following in mg/kg diet: NaCl, 5000; KI, 6; $MnSO_4.H_2O$, 65; $Fe_2SO_4.7H_2O$, 300; $CuSO_4.5H_2O$, 7; $CoCl_2.6H_2O$, 20; $ZnSO_4.7H_2O$, 15; alpha floc as carrier.
[c]Analyzed results are expressed as percentage of dry matter (except moisture).

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Table 2 Formulation of dietary pretreatment meals

	Pretreatment 1				Pretreatment 2			
	PCPA		placebo		tryptophan		placebo	
	(HP)	(HC)	(HP)	(HC)	(HP)	(HC)	(HP)	(HC)
Ingredients (% inclusion)								
Fishmeal	48.4	33.2	48.4	33.2	48.4	33.2	48.4	33.2
Soybean meal	30.4	20.9	30.4	20.9	30.4	20.9	30.4	20.9
Vitamin premix[a]	2	2	2	2	2	2	2	2
Mineral premix[b]	1	1	1	1	1	1	1	1
Fish oil	8.17	4.51	8.17	4.51	8.17	4.51	8.17	4.51
Soybean oil	0.17	—	0.17	—	0.17	—	0.17	—
Glucose	—	28.5	—	28.5	—	28.5	—	28.5
Pretreatment premix								
D,L-PCPA	5.00	5.00	—	—	—	—	—	—
L-tryptophan	—	—	—	—	7.00	7.00	—	—
L-alanine	3.86	3.86	6.11	6.11	—	—	6.11	6.11
chromic oxide	1.00	1.00	1.00	1.00	—	—	—	—
carmine	—	—	—	—	0.85	0.85	0.85	0.85
cellulose	—	—	2.75	2.75	2.01	2.01	2.90	2.90

[a]Vitamin premix supplied the following in mg/kg diet (except where units are noted): vitamin A (retinyl palmitate and retinyl acetate), 4165 IU/kg; vitamin D_3, 835 IU/kg; vitamine E (dl-alpha-tocopheryl acctate), 300; vitamin K (menadione sodium bisulphite), 25; thiamin mononitrate, 10; riboflavin, 10; niacin, 99; pyridoxine HC1, 40; D-Ca pantothenate, 100; folic acid 17; biotin 0.5; cyanocobalamin, 0.2; choline chloride, 5500; ascorbic acid, 1000; butylated hydroxytoluene, 25; DL-methionine, 3000; alpha floc as carrier.
[b]Mineral premix supplied the following in mg/kg diet: NaCl, 5000; KI, 6; $MnSO_4.H_2O$, 65; $Fe_2SO_4.7H_2O$, 300; $CuSO_4.5H_2O$, 7; $CoCl_2.6H_2O$, 20; $ZnSO_4.7H_2O$, 15; alpha floc as carrier.

sized pellets using a Moulinex meat grinder (Model 244, Canadian Tire, Guelph, Ontario, Canada). Pellets were dried in a forced-air drying oven and stored at −20° C until required.

Supply and maintenance of fish

Juvenile rainbow trout (initial body weight 3450 ± 120 [SD] g/tank (114.3 g/fish), Spring Valley Trout Farm Ltd, Thamesford, Ontario, Canada) were randomly distributed into 24 dark tan 60 L fiberglass tanks (30 fish/tank). The water, which was aerated continuously and maintained at 15° C in a flow-through system, was a mixture of approximately 50% City of Guelph water and 50% artesian well water. Tanks were housed in a windowless laboratory; photoperiod was maintained at 12 hours light (07:00 hr–19:00 hr) and 12 hours dark (19:00 hr–07:00 hr). Fish were fed the appropriate diet (HP or HC) to satiety 3 times/day (09:00 hr, 13:00 hr, 17:00 hr), 7 days/week for 6 weeks.

Sampling of fish

Pre-experiment trials showed that changes in brain 5-HT turnover, as assessed by the concentration of 5-HIAA or the ratio of 5-HIAA/5-HT,[24] could be produced by pretreatment with 5% dietary PCPA (one meal to satiety, 72 hours before sampling) or 7% dietary crystalline tryptophan (one meal to satiety, 24 hours before sampling). Dietary pretreatment meals were provided to fish in a HP or HC formulation consistent with the diet on which they had been raised *(Table 2)*. Fish received two dietary pretreatment meals prior to sampling, as outlined in *Figure 1*. Fish that received tryptophan (TRP) as the second dietary pretreatment meal received either PCPA (PCPA pretreatment) or placebo (TRP pretreatment) as the first dietary pretreatment meal. This was done to dissociate altered food intake due specifically to increased 5-HT from altered consumption resulting from increased tryptophan.

Following the 6-week acclimation period, fish were fasted for 3 days to ensure complete evacuation from the stomach of residual food.[15,25,26] To ensure that fish were sampled within a fairly narrow time frame (09:00 hr–12:00 hr), sampling was conducted on four consecutive days. At 09:00 hr on the morning of sampling, fish were fed the appropriate test meal (HP or HC) to satiety. Eight fish/tank were removed and immediately anesthetized in a solution of tricaine methane sulfonate (300 mg/L). On each fish the spinal cord was severed and the brain immediately removed, frozen in liquid N_2, and stored at −80° C until analysis. Stomachs were ligated as previously described.* Fish were frozen at −20° C until separation and quantification of stomach contents.

Tissue analysis

Tryptophan, 5-HT, 5-HIAA, and 5-HIAA/5-HT of individual fish brains were determined by a modification of the HPLC-EC method of Mefford[27] as previously described.[28] Stomach contents were removed, separated into red, green (dietary pretreatment meal), and brown (test meal) components, quantitated by weighing, and expressed as dry content weight as a percent of wet body weight.*

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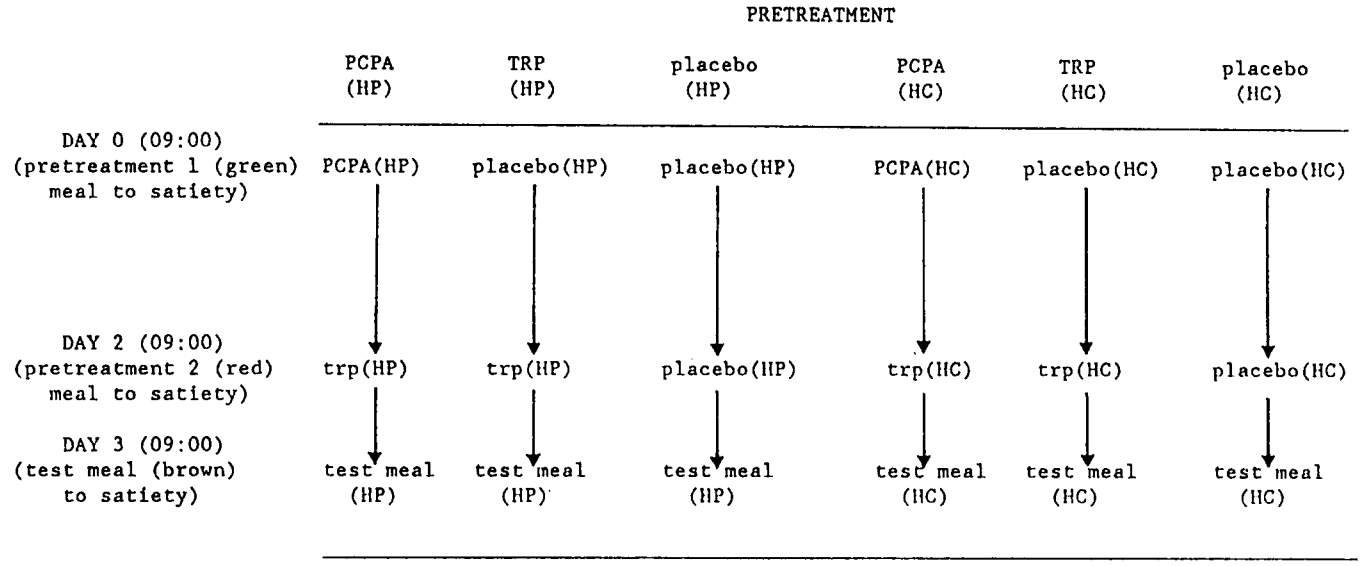

Figure 1 Application of dietary pretreatments and test meal.

Statistical analysis

The experiment was conducted using a 2 × 3 factorial design (two diets, three pretreatments) with four replicates (tanks of fish)/treatment.[29] Data obtained on brain tryptophan, 5-HT, 5-HIAA, 5-HIAA/5-HT, residual stomach contents, and test-meal stomach contents were analyzed by analysis of variance for a 2 × 3 factorial design.[29] Significance of differences of the main effects were tested at the 5% level. The effect of pretreatment was determined by contrasting PCPA with placebo or TRP with placebo, and the effect of diet by comparison of HP and HC. Interaction was tested at the 15% level† and when interaction was detected, the appropriate simple effects were tested as outlined for main effects.

Results

Interaction between diet and pretreatment was noted for brain tryptophan concentration ($P \leq 0.007$, *Figure 2A*). Analysis of simple effects showed that for fish fed the HP diet, neither PCPA nor TRP pretreatment resulted in elevated brain tryptophan at the time of sampling. However, for fish fed the HC diet, brain tryptophan was elevated in the TRP pretreatment group. Further analysis of simple effects showed that in fish pretreated with TRP, brain tryptophan was lower in those fed the HP diet than in those fed the HC diet.

For brain 5-HT there was no interaction between diet and pretreatment ($P \geq 0.726$, *Figure 2B*). Examination of main effects indicated no difference between the two diets with regard to brain 5-HT following the different pretreatments. However, regardless of diet, brain 5-HT was lower in fish pretreated with PCPA than in the placebo group. Pretreatment with

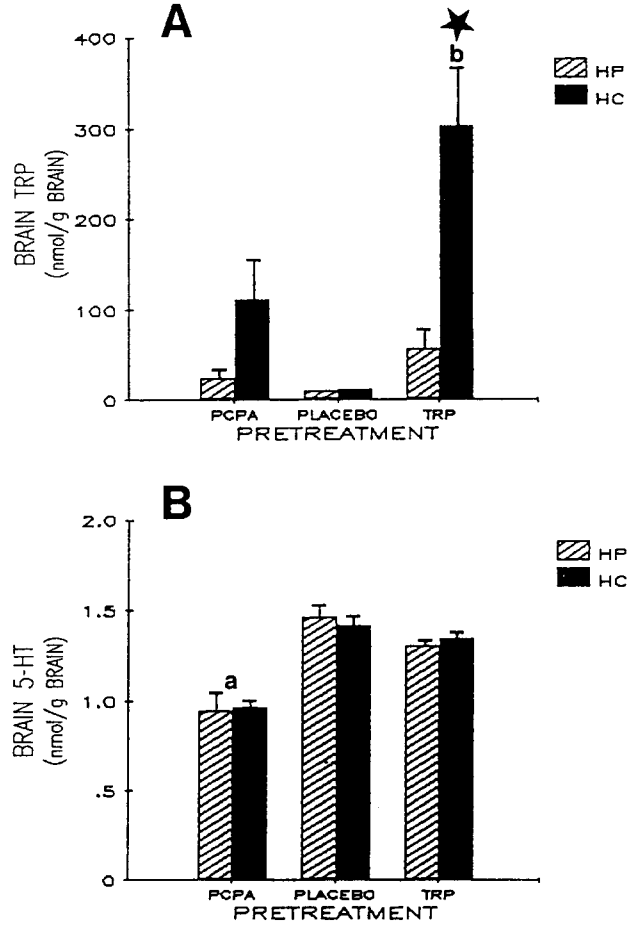

Figure 2 Effect of pretreatment and diet on brain tryptophan and 5-HT concentrations in rainbow trout. Each bar represents the mean of four replicates (eight fish/replicate). Error bars represent + 1 SEM. A) Brain Trp–Interaction detected ($P < 0.15$). Simple effects–★ TRP (HC) higher than TRP (HP) ($P < 0.05$); [b]TRP (HC) higher than placebo (HC). B) Brain 5-HT–No interaction detected ($P > 0.15$). Main effect–[a]PCPA lower than placebo ($P < 0.05$).

†Allen, O.B. Associate Professor, Department of Mathematics and Statistics, University of Guelph, Guelph, Ontario, Canada. Personal communication.

TRP did not affect brain 5-HT. For brain 5-HIAA, no interaction was detected ($P \geq 0.724$, *Figure 3A*). Analysis of main effects indicated no differences between diets regardless of pretreatment. However, regardless of diet, PCPA pretreatment decreased, and TRP pretreatment increased, brain 5-HIAA. For the brain ratio of 5-HIAA/5-HT, no interaction was detected ($P \geq 0.823$, *Figure 3B*), and there was no effect of diet. However, regardless of diet, fish pretreated with TRP showed an increased ratio compared with fish pretreated with placebo, while the ratio was similar to the placebo group in fish pretreated with PCPA.

Quantitation of residual stomach contents (comprised only of the second pretreatment meal; no first pretreatment meal remained in any of the fish) showed no interaction between diet and pretreatment ($P \geq 0.588$, *Figure 4A*) and no effect of pretreatment. However, regardless of pretreatment, fish fed the HC diet had more residual food in the stomach than fish fed the HP diet. Analysis of test meal stomach contents revealed an interaction between diet and pretreatment

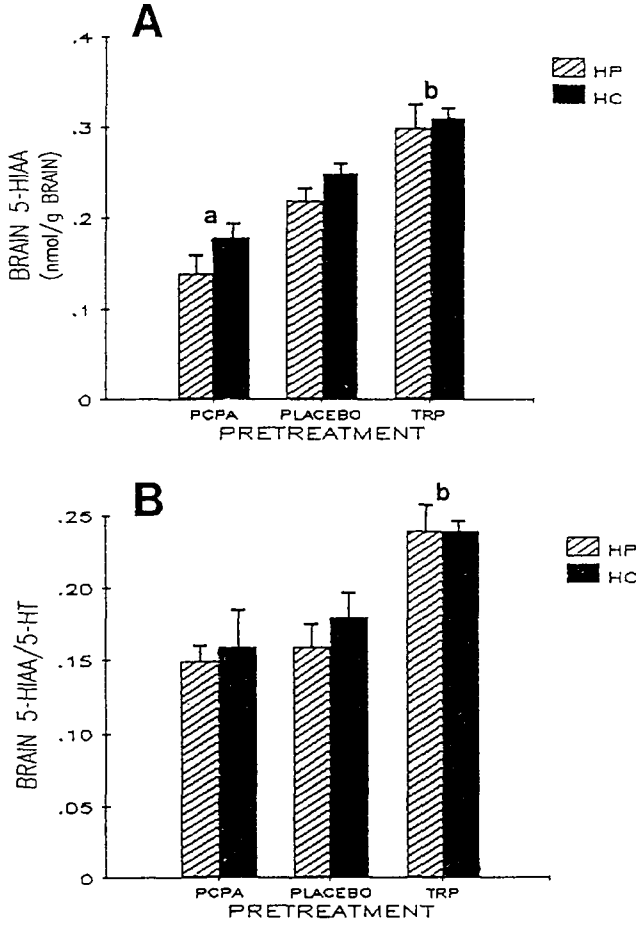

Figure 3 Effect of pretreatment and diet on brain 5-HIAA and 5-HIAA/5-HT in rainbow trout. Each bar represents the mean of four replicates (eight fish/replicate). Error bars represent + 1 SEM. A) Brain 5-HIAA–No interaction detected ($P > 0.15$). Main effects–[a]PCPA lower than placebo ($P < 0.05$); [a]TRP higher than placebo ($P < 0.05$). B) Brain 5-HIAA/5-HT–No interaction detected ($P > 0.15$). Main effect–[b]TRP higher than placebo ($P < 0.05$).

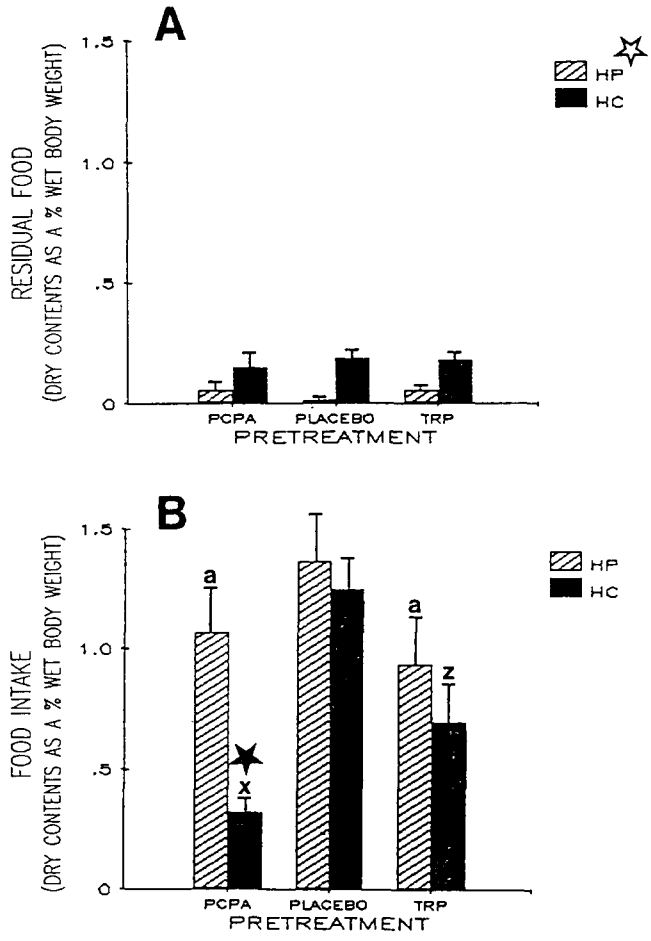

Figure 4 Effect of pretreatment and diet on residual stomach contents and test meal food intake in rainbow trout. Each bar represents the mean of four replicates (eight fish/replicate). Error bars represent + 1 SEM. A) Residual stomach contents–no interaction detected ($P > 0.15$). Main effect–☆ HC higher than HP ($P < 0.05$). B) Test meal food intake–interaction detected ($P < 0.15$). Simple effects–[x]PCPA (HC) lower than placebo (HC); [z]TRP (HC) lower than placebo (HC) ($P < 0.05$); ★PCPA (HC) lower than PCPA (HP) ($P < 0.05$). [a]not different from placebo (HP diet) ($P > 0.05$).

($P \leq 0.146$, *Figure 4B*). Examination of simple effects showed no effect of pretreatment in fish fed the HP diet. However, in fish fed the HC diet, both PCPA and TRP pretreatment decreased food intake. Finally, examination of pretreatment simple effects showed that food intake of PCPA-pretreated fish was lower in fish fed the HC diet than in those fed the HP diet. No further pretreatment simple effects were noted.

Discussion

Dietary pretreatment with a meal containing PCPA or tryptophan was used to manipulate brain 5-HT in rainbow trout to address the putative role of this neurotransmitter in controlling intake of either a typical, high protein, low-CHO diet, or of an economically preferable high-CHO diet. PCPA was selected to decrease 5-HT for two reasons. First, because it inhibits

5-HT synthesis, quantification of 5-HT and 5-HIAA allowed titration of a dosage that altered brain 5-HT turnover without causing overt signs of toxicity. Second, PCPA has previously been shown to decrease 5-HT levels in the brain of a closely related fish (the chum salmon) when administered chronically in the diet.[30] Tryptophan loading was chosen as a method to increase 5-HT turnover because we previously demonstrated that chronic dietary tryptophan supplementation produced easily measured, parallel changes in brain tryptophan, 5-HT, and 5-HIAA.[28]

Pretreatments were administered in the diet. This was done to minimize non-specific, stress-induced alterations in food intake, which might accompany other techniques such as gastrointestinal intubation, intraperitoneal injection, or direct injection into the ventricles of the brain. However, because most of the body's 5-HT is present in tissues peripheral to the brain, especially in the gastrointestinal tract, this has the potential disadvantage of altering gastric emptying, thereby resulting in altered amounts of residual food in the stomach.[31,32] This may affect subsequent food intake and thereby complicate assessment of the role of brain 5-HT in mediating satiety. Because sequential meals do not mix to any extent in the stomach of rainbow trout,* colored diets can be used to distinguish residual and test meal contents. This allows direct measurement of the extent to which this may be a confounding factor. In this experiment, quantitation of residual stomach contents indicated no effect of pretreatment; PCPA-, TRP-, and placebo-pretreated fish had similar amounts of food remaining. This suggests that any differences in test meal consumption cannot be attributed to variable amounts of residual food. While dietary pretreatment had no effect on residual food, fish fed the HC diet had more residual stomach contents than did those fed the HP formulation. This could be explained either by greater consumption of the second pretreatment meal by HC fish or by delayed emptying from the stomach. It is likely that this primarily reflects delayed gastric emptying because pretreatment meal consumption appeared similar in the HP and HC groups. In addition, consumption of the test meal by the placebo-pretreated HP and HC fish was not different and, if anything, appeared lower in HC fish. However it should be noted that Jobling[33] was unable to detect an effect of nutrient type on gastric evacuation in plaice. Therefore, to answer this question directly, it would be necessary to conduct gastric evacuation studies following consumption of high-CHO or high-protein diets by trout.

Although both the PCPA and TRP groups received 7% tryptophan as the second pretreatment meal, brain tryptophan levels differed. In the HP group, brain tryptophan was similar to placebo in both the PCPA- and TRP-pretreated groups. However, for the TRP-pretreated group, fish fed the HP diet had a significantly lower brain tryptophan than those fed the HC formulation. This may reflect delayed gastric emptying in the HC group because the second pretreatment meal was probably still being absorbed from the gastrointestinal tract. In addition, the metabolic changes that are typical of trout raised on high-CHO diets[17,19] may have interfered with metabolism of the tryptophan load, thereby leading to its accumulation. The reason why the PCPA (HC) group did not show the same elevation in brain tryptophan as the TRP (HC) group is unknown. It is possible that consumption of the 7% tryptophan meal was lower in the PCPA group as these fish had already received PCPA 2 days earlier and this agent decreases food intake by these fish. While at first the residual food data seem to argue against this possibility (similar amounts of residual food being present in TRP and PCPA groups), delayed gastric emptying in the PCPA group may reconcile these findings. In particular, previous studies indicated that gastric emptying was complete in 36 hours in fish fed 1%, 2%, or 3% tryptophan and 0.1% PCPA but was not complete until 48 hours in fish fed 0.3% and 1% PCPA.‡ Therefore, delayed gastric emptying, together with decreased consumption of the second meal, probably explains the difference in response of brain tryptophan in the PCPA (HC) and TRP (HC) groups.

Analysis of brain 5-HIAA and 5-HIAA/5-HT confirmed that PCPA decreased brain 5-HT turnover (decreased 5-HIAA) and TRP increased 5-HT turnover (increased 5-HIAA and 5-HIAA/5-HT).[24] Furthermore, diet had no effect on brain 5-HT turnover as the changes resulting from the pretreatment were almost identical for fish fed either the HP or HC diet. In fish fed the HP diet, test meal consumption was not affected by pretreatment either with PCPA or TRP, even though these pretreatments altered brain 5-HT turnover. This suggests that in trout fed a high protein, low digestible CHO diet, brain 5-HT does not control food consumption. These results agree with previous results of this laboratory,[28] in which chronic increases in dietary tryptophan, above the requirement level, resulted in parallel elevations in brain 5-HT and 5-HIAA, with no concomitant change in chronic food intake or weight gain. In that experiment, the semi-purified diets used were also high in protein and low in digestible CHO.

Pretreatment had a similar effect on brain 5-HT in fish fed both the HC and HP diet. However, test meal intake was affected differently. That is, while consumption of the test meal was unaffected by pretreatment in the HP fish, pretreatment with either PCPA or TRP decreased intake by HC fish. This occurred even though the PCPA and TRP pretreatments had opposite effects on brain 5-HT turnover. Several explanations for this observation are possible. For ex-

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‡Johnston, W.L. and Glanville, N.T. Unpublished observation.

ample, one possibility is that both the PCPA and tryptophan effects were brain 5-HT–mediated. This would imply that brain 5-HT plays a dual role in mediating CHO consumption, with both high and low levels causing decreased consumption of a HC diet. While this seems paradoxical, it is possible that different levels of brain 5-HT activate different receptor classes, for example an autoreceptor and a post-synaptic receptor, which mediate different events. A similar explanation has been proposed to explain why, depending on the dosage used, 8-OH-DPAT either increases or decreases food intake.[34,35] Additional studies using pharmacologic agents with selectivity for different 5-HT receptor classes may be helpful to further investigate this possibility in trout.

Another possible explanation is that for both the pretreatments, the decreased food intake was independent of the changes in brain 5-HT turnover. A limitation of using pharmacological agents to manipulate brain 5-HT is that these drugs may have additional, non–5-HT effects, and in high doses may be toxic. While the trout did not show overt signs of malaise (fish were active and vigorously approached the surface of the water when the tanks were opened, and there were no mortalities or fish lying on the bottom of the tanks), more subtle behavioral changes could have precipitated decreased food consumption. In the case of tryptophan loading, this may result in elevated synthesis of other neuroactive compounds derived from tryptophan. For example, in rats, the striatal extracellular fluid concentration of quinolinic acid, an N-methyl-D-aspartate (NMDA) receptor agonist, is dramatically elevated following intraperitoneal tryptophan loading.[36] Therefore, an elevation of quinolinic acid or other neuroactive compounds derived from tryptophan (for example, tryptamine[37] or kynurenic acid, an NMDA antagonist[38]) may explain the decreased food intake of the TRP-pretreated (HC) group. Elevation of non–5-HT, tryptophan-derived compounds cannot account for the diminished intake in the PCPA-pretreated (HC) group because additional experiments showed that intake of the HC diet by trout pretreated with 5% dietary PCPA was decreased to the same degree, relative to control, in trout fed either 7% tryptophan or alanine (placebo) as the second dietary pretreatment meal. However, 5-HT–independent PCPA-specific effects may have been responsible for the diminished food intake§. Why brain 5-HT–independent or toxic effects would affect consumption only of the HC diet is unclear. It is possible that the metabolic changes apparent in trout raised on high CHO diets could exacerbate the pretreatment effects. In particular, pathological changes in liver enzyme activity and glycogen storage (hepatomegaly),[17,19] which result when trout are fed a high CHO diet, may impair the ability of these animals to detoxify PCPA or excess tryptophan. A similar explanation has been proposed to explain why trout fed high CHO diets are more susceptible to Cu or Se toxicity.[39,40]

A further possibility is that, for only one of the treatments, decreased food intake was brain 5-HT–specific while for the other, it was brain 5-HT–independent. For example, it is possible that the PCPA effect was specifically mediated by a brain 5-HT system that inhibits CHO satiety, while the effect of TRP was 5-HT–independent. Conversely, the TRP effect may have derived from a brain 5-HT system that enhances CHO satiety, while the effect of PCPA was independent of this system. If the trout is similar to other vertebrates, it might be predicted that the tryptophan effect is brain 5-HT–specific, while that of PCPA is independent of this system, because in rats, for example, 5-HT has been proposed to mediate CHO satiety. However, we have previously demonstrated that food consumption decreases brain 5-HT turnover in trout relative to fasted fish.[15] Furthermore, brain tryptophan, 5-HT, 5-HIAA, and 5-HIAA/5-HT were similar in HP and HC placebo groups. In addition, Sloley et al.[41] and Walton[19] found that brain levels of tryptophan and 5-HT were similar in fish fed either high protein, high CHO or high lipid diets. Therefore, this suggests that intake of either HC or HP diets probably results in similar decreased brain 5-HT turnover compared with fasted fish. Therefore, food consumption in trout fed a high CHO diet, rather than increasing brain 5-HT, is likely to decrease brain 5-HT, relative to fasting, which is opposite to what has been proposed to occur in a 5-HT CHO satiety feedback loop in mammals. If, instead of tryptophan, it is the PCPA effect that is mediated specifically by (lower) brain 5-HT, this would suggest that a system for controlling CHO intake exists in the carnivorous rainbow trout that is opposite to that proposed to operate in omnivorous vertebrates such as the rat or the human. While this is possible, more evidence would be required before this could be considered a likely explanation.

In summary, the results of this experiment show that when trout are fed a high protein, low CHO diet, brain 5-HT does not appear to play a role in controlling food intake. A role in specifically controlling CHO intake in trout cannot be completely excluded by these data. However, both increases and decreases in brain 5-HT turnover were associated with depressed intake of the HC diet, suggesting that pretreatment effects may be 5-HT–independent. Additionally, HP and HC diets had similar effects on brain 5-HT turnover, arguing against the presence of a feedback loop. Furthermore, because trout do not normally consume much digestible CHO, this suggests that brain 5-HT is also unlikely to mediate CHO consumption.

§Johnston, W.L. and Glanville, N.T. Effect of PCPA on food intake and ^{3}H-5-HT binding in rainbow trout, *Oncorhynchus mykiss*. Manuscript in preparation.

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