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5-Hydroxy-L-tryptophan suppresses food intake in food-deprived and stressed rats

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Abstract

Giving L-tryptophan, serotonin's circulating precursor, or a serotonin-releasing drug can decrease food intake and body weight. Giving 5-hydroxy-L-tryptophan (5-HTP), serotonin's immediate intracellular precursor, has been thought to be ineffective in enhancing brain serotonin synthesis unless it is coadministered with a dopa decarboxylase inhibitor to protect 5-HTP from destruction outside the brain. We have examined the effect of 5-HTP on food consumption and tissue 5-HTP levels among rats subjected to two different hyperphagic stimuli, food deprivation and a standardized stress (tail pinch), and on plasma 5-HTP levels in humans. In rats, 5-HTP (3–200 mg/kg ip) suppressed food intake in a dose-dependent manner in both models, but was at least eight times more effective in our stress–hyperphagia model. (Differences in the two procedures might have contributed to the observed differences in potencies.) This suppression was blocked by coadministration of another large neutral amino acid (LNAA), L-valine. Brain 5-HTP levels correlated significantly with peak plasma 5-HTP (r^2=.69) or 5-HTP/LNAA (r^2=.81) levels. Additionally, among humans, oral 5-HTP (1.2–2.0 mg/kg) produced, after 1 and 2 h, a significant increase in plasma 5-HTP (1.5- to 2.3-fold). These observations suggest that 5-HTP may be useful in controlling the excessive food intake sometimes generated by stress, even if given without decarboxylase inhibitors or other drugs.

Keywords: 5-Hydroxy-L-tryptophan; Serotonin; Food intake; Protein; Carbohydrate

1. Introduction

The large neutral amino acid (LNAA), serotonin precursor, 5-hydroxy-L-tryptophan (5-HTP), has been used experimentally in the past to treat depression (Van Praag, 1981), schizophrenia (Wyatt et al., 1972), and myoclonus (Van Woert and Rosenbaum, 1979). It has been assumed that its rapid peripheral metabolism by L-amino acid decarboxylase (L-AAAD) limits the amount available to enter the brain so that 5-HTP must be given with a L-AAAD inhibitor (Van Woert and Rosenbaum, 1979). Since the release of brain serotonin is known to affect satiety and macronutrient (protein vs. carbohydrate) selection, oral 5-HTP might affect feeding behaviors, and thus body weight. While peripheral administration of serotonin fails to cross the blood–brain barrier, administration of the amino acid precursor 5-HTP would be expected to enter the brain and presumably affect brain serotonin-mediated functions.

The administration of 5-HTP to nonfasted control rats (25–100 mg/kg ip) has been reported to cause a significant dose-dependent decrease in 24-h food intake (Ju and Tsai, 1995). In this study, the authors also demonstrated that the administration of the nonselective serotonin receptor antagonist cyproheptadine, or the semiselective serotonin-1 and -2 receptor antagonists propranolol and sulpiride, were capable of partially antagonizing the anorectic effect of administered 5-HTP. Another study by Fletcher and Burton (1986) demonstrated the anorectic effects of 5-HTP in a 1-h feeding test in food-deprived rats. In this study, both serotonin and 5-HTP exhibited anorectic activity that was antagonized partially by the peripheral serotonin receptor blocker xylamidine, thus suggesting a partial peripheral component to their anorectic actions. In the same study, the anorectic activity of the serotonin reuptake and releasing

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agent fenfluramine was found not to be influenced by the peripheral blockade of serotonin receptors.

In humans, administration of 5-HTP (8 mg/kg/day) to obese female subjects was found to decrease carbohydrate and total calorie intake during a 5-week placebo-controlled trial (Ceci et al., 1989). In another study, similar results were found when 5-HTP (900 mg/day) was administered to obese subjects for 6 weeks (Cangiano et al., 1992). Similarly, Cangiano et al. (1998) demonstrated in a placebo-controlled, double-blind clinical trial that 5-HTP (750 mg/day) could preferentially decrease carbohydrate intake in patients with non-insulin-dependent diabetes mellitus.

In the current study, we have explored the ability of 5-HTP to suppress food consumption in fasted and mildly stressed rats and to alter plasma and brain levels of this amino acid. Additionally, since 5-HTP is also available for human use in the form of various dietary supplements, for comparison, we also determined the ability of low doses of 5-HTP, given without a peripheral decarboxylase inhibitor, to increase blood 5-HTP in humans. (In most prior studies, human subjects have been given a peripheral decarboxylase inhibitor along with 5-HTP, on the assumption that the drug is needed if blood 5-HTP levels are to rise.) We find that 5-HTP suppresses food intake by rats, and that the effect of 5-HTP on food intake in fasting rats is suppressed by the coadministration of L-valine, an amino acid that, like 5-HTP, also enters the brain via the LNAA transport system (Maher et al., 2001). Moreover, even in the absence of an L-AAAD inhibitor, low doses do raise plasma 5-HTP in humans.

2. Materials and methods

2.1. Animal studies

All experimental protocols were approved by the MCPHS Institutional Animal Care and Use Committee (IACUC) prior to commencement of the studies. For studies on effects of 5-HTP on food intake in food-deprived rats, male Sprague–Dawley rats, 125–150 g at the start of the experiments (Charles River Laboratories, Wilmington, MA), were housed individually in suspended wire mesh cages, maintained on a reversed lighting schedule (lights on at 7 p.m., lights off at 7 a.m.), and acclimated to our climate-controlled animal facility and to the test diets for 5 days prior to experimentation. As the experiments in food-deprived rats were performed during the dark cycle, when rats normally eat, a dim red light (25 W) was used to help with placement and weighing of the food jars. Since the time of daily lighting exposure was reversed (for the convenience of the investigators), a re-entrainment period of 5 days was included.

For studies on the effects of the 5-HTP on stress-induced eating, animals that had free access to food and water were maintained in polycarbonate cages with stainless steel lids and exposed to a standard 12-h light/dark cycle (lights on at 7 a.m., lights off 7 p.m.) and experiments were performed during the lights on phase.

2.2. Diet and treatments

Rats had ad libitum access to tap water and food (Rodent Laboratory Chow No. 5001 pellet form, Purina) on non-testing days. A mash diet, used for experiments on food-deprived animals, and composed of equal parts by weight of ground rodent chow (No. 5001 meal form, Purina) and a 4% nutrient agar solution (Teklad Diets), was presented to the animals in glass jars. The use of this agar-based mash diet minimizes spillage and allows for more accurate measurement of food intake. Additionally, it has been shown previously that this mash diet is sufficient for maintaining normal rat growth (Hull and Maher, 1990).

5-HTP, 98% purified from the plant source *Griffonia simplicifolia* (Laboratoire Oenobiol, Paris, France) and L-valine (Sigma-Aldrich, USA), were suspended in 0.9% saline and injected intraperitoneally in a volume of 5 ml/kg body weight.

2.3. Experimental procedures

2.3.1. Effects of 5-HTP on food intake in food-deprived rats

Four groups of male Sprague–Dawley rats ($n=14$) were used in this experiment. On the day of the experiment, food was removed at 7 a.m. prior to lights off; 4 h later (11 a.m.), 5-HTP (0, 50, 100, 200 mg/kg) was administered intraperitoneally. One hour later, preweighed food jars were introduced into the cages and a sheet of clean white paper was placed under each cage to collect any spillage. The amounts of food consumed after 1, 2, and 4 h were determined to the nearest 0.01 g.

2.3.2. Effect of LNAA, L-valine, on the food intake of food-deprived rats treated with saline or 200 mg/kg of 5-HTP

Food was removed at 7 a.m., prior to lights off, as described above, and 4 h later, L-valine, 5-HTP, and/or saline were coadministered intraperitoneally to four groups of rats ($n=4$) as follows: saline/saline (control), saline/L-valine (300 mg/kg), saline/5-HTP (200 mg/kg), and L-valine (300 mg/kg)/5-HTP(200 mg/kg). The dose of L-valine was chosen based on previous studies in which this LNAA has been used to competitively inhibit the transport of other LNAA across the blood–brain barrier (Hull and Maher, 1990). One hour later, preweighed food jars were introduced and the amounts of food consumed after 1, 2, and 4 h were determined as described above.

2.3.3. Effect of 5-HTP on stress-induced eating

Tail pinch has been used to stimulate eating and other stress-related behavioral activities in rats (Clark et al., 1992). Application of an unavoidable peripheral stressor (like tail pinch) produces a profile of behaviors including eating, gnawing, and licking. This response may have interesting

parallels with stress-induced eating in humans (Clark et al., 1992; Czech et al., 1998; Morley and Levine, 1980).

On a pretest day, naïve animals were individually adapted for 10 min to a new cage environment that included having 10 weighed chow pellets on the floor of the cage. After the adaptation period, a 10-in. hemostat with foam rubber padded tips was used to apply tail pinch; it was placed 1 in. from the tip of the animal's tail and locked on the first notch. Animals received six 20-s tail pinches; each separated by 5–8 min. Animals were observed for evidence of eating behaviors. Animals that did not eat in response to tail pinches were excluded from further study.

On a subsequent test day, seven groups of rats ($n=6–8$) received 5-HTP (3, 6, 12, 25, 50, 100, and 200 mg/kg ip in saline), and were housed singly in standard cages for 1 h with no bedding and without access to food or water. One hour later, the animals were tested for appetitive behavior as described above for the pretest period. Animals continued to have access to chow for 5 min after the final tail pinch; food intake was measured to the nearest 0.01 g. Each animal received vehicle ($n=18$) as a treatment for the determination of its individual baseline feeding value. At least 3 days separated each experiment, and animals were rotated to ensure that no individual animal received the same treatment twice, and scheduled so that the order of treatments was randomized. These animals were only used in the tail pinch experiments (and not the above food-deprivation studies) and in three or fewer tail pinches trials (involving vehicle and no more than two 5-HTP doses).

2.3.4. 5-HTP and other LNAA in plasma and brain

Four groups of male Sprague–Dawley rats ($n=6$) were used in this experiment. On the day of the experiment, food was removed prior to lights off, and 5-HTP (0, 50, 100, and 200 mg/kg) was administered intraperitoneally 4 h later. After 1 h, rats were sacrificed via carbon dioxide asphyxiation, and blood was collected and brains removed rapidly and frozen. Blood was collected in sodium heparin, and plasma separated and frozen at −70 °C until chemical analysis. For LNAA determination, plasma was deproteinized with perchloric acid, filtered (0.22 um) and assayed using high-performance liquid chromatography.

Whole brains were extracted using a modification of the technique of Glaeser et al. (1983). Following homogenization in 5% trichloroacetic acid (Fischer Scientific, Fair Lawn, NJ) and liquid phase extraction with diethyl ether (J.T. Baker, Phillipsburg, NJ), samples were analyzed for LNAA using high-performance liquid chromatography with fluorometric detection, according to the method of Berardino et al. (1990).

2.4. Human studies

All human studies had approval of the MIT Committee on the Use of Humans as Experimental Subjects prior to commencement, and were conducted within the MIT Clinical Research Center. To determine the pharmacokinetic profile of 5-HTP in man, we recruited six healthy female subjects of mean age 26.8 years and mean body mass index 23.7. Following a physical examination and confirmation of informed consent, each subject received per os in a cross-over design 100 or 150 mg 5-HTP. This approximated a dose of 1.2–2.0 mg/kg. On the morning of the experiment at approximately 8 a.m., after an overnight fast, blood (2 ml) was taken from each subject. Subjects then received, in a dose-randomized fashion, 5-HTP in capsule form. Blood was again collected at 1, 2, 4, 8, and 24 h post 5-HTP administration. Half hour later and again after the last blood draw, each subject received a small mixed carbohydrate/protein meal. Plasmas were separated from whole blood and stored for subsequent analyses (as described above).

2.5. Data and statistical analyses

In the food deprivation experiment, cumulative food intake (grams of food/100 g of rat body weight) is expressed as group means ± S.E.M. Differences between groups were determined using independent one-way ANOVA and post hoc Newman–Keuls testing (minimum significance level was set at $P<.05$), with separate analyses performed at each measurement time point.

In the stress-induced eating experiment, comparisons were made between the percentages of the food each rat consumed after treatment and its control value (the food consumed by the same rat when treated with the vehicle and exposed to the same procedure of tail pinch).

Differences in plasma and brain levels of 5-HTP in rats were analyzed using independent ANOVAs and post hoc Newman–Keuls testing. Differences in plasma levels of 5-HTP in humans was determined using ANOVA with repeated measures and post hoc Newman–Keuls testing.

3. Results

3.1. Animal studies

3.1.1. Effect of 5-HTP on food intake by food-deprived rats

Intraperitoneally administered 5-HTP caused a significant dose-related attenuation of food intake in fasted rats (Fig. 1); the ANOVAs yielded $F(3,52)=13.84$, 12.63, and 11.17 (all $P<.001$) at 1, 2, and 4 h, respectively. When compared to vehicle control condition, both 100 and 200 mg/kg of 5-HTP significantly reduced food intake at all time points ($P<.05$ or .001) as determined by Newman–Keuls test. The 50-mg/kg dose of 5-HTP failed to reach statistical significance ($P>.05$).

3.1.2. Effect of the LNAA L-valine on 5-HTP-induced decreases in food intake by food-deprived rats

L-Valine (300 mg/kg) given alone failed to alter food intake. However, it significantly attenuated the ability of 5-

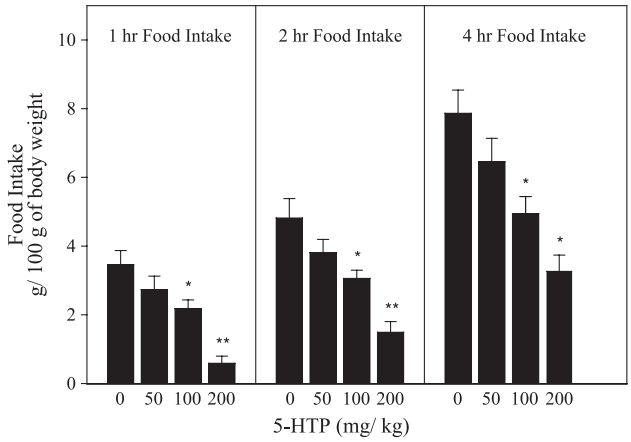

Fig. 1. Effect of 5-HTP administration on food intake in food-deprived rats: mean ± S.E.M. cumulative food intake at 1, 2, and 4 h in 4-h fasted rats intraperitoneally administered with various doses of 5-HTP. * $P<.05$, ** $P<.001$, when compared to the vehicle-treated group (saline control) as determined by ANOVA and post hoc Students–Newman–Keuls test (group ns = 14).

HTP (200 mg/kg) to reduce food intake in fasted rats (Fig. 2); the ANOVAs yielded $F(3,12) = 12.29$, $P<.001$; $F(3,12) = 6.52$, $P<.05$; $F(3,12) = 6.06$, $P<.05$, at 1, 2, and 4 h, respectively. When compared to control condition (the group treated with only 5-HTP), L-valine significantly attenuated the anorectic effect of 5-HTP at all time points ($P<.05$) as determined by Newman–Keuls test.

3.1.3. Effect of 5-HTP on stress-induced eating

5-HTP significantly and dose-dependently decreased food intake among stressed rats (Fig. 3); the ANOVA yielded $F(7,58) = 12.16$, $P<.001$. When compared to vehicle control condition, 12, 25, 50, 100, and 200 mg/kg of 5-HTP significantly reduced food intake ($P<.05$) as determined by Newman–Keuls test. The 3- and 6-mg/kg doses of 5-HTP failed to affect stress-induced eating significantly ($P>.05$).

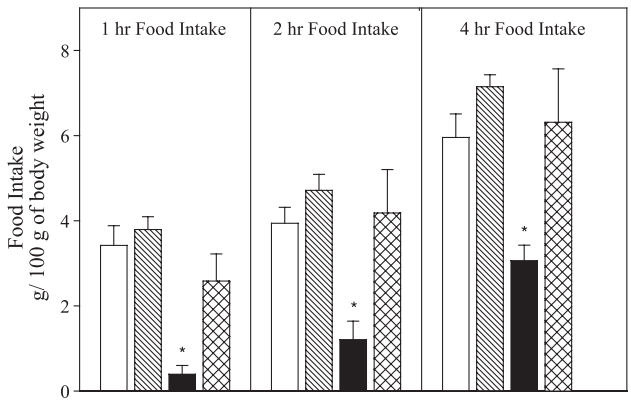

Fig. 2. Effect of L-valine on reduction in food intake by 5-HTP in food-deprived rats: mean ± S.E.M. cumulative food intake at 1, 2, and 4 h in 4-h fasted rats after intraperitoneal coadministration of L-valine (300 mg/kg) and 5-HTP (200 mg/kg). Four groups of rats were administered the combination of saline–saline □, L-valine–saline ▨, 5-HTP–saline ■, or 5-HTP–L-valine ▩. * $P<.05$, when compared to the other groups as determined by ANOVA and post hoc Students–Newman–Keuls test (group ns = 4).

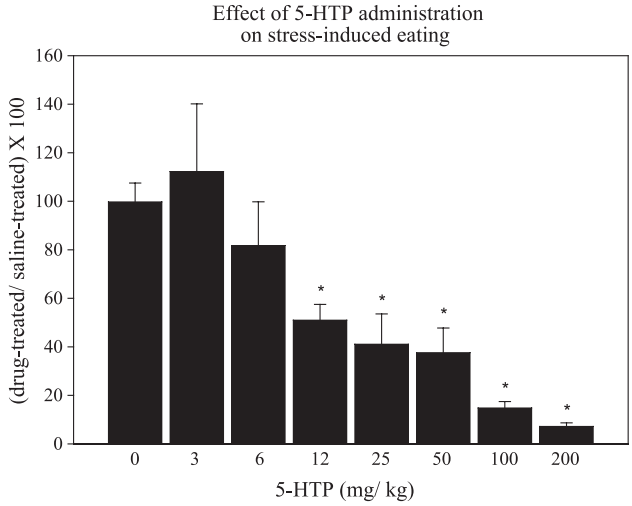

Fig. 3. Effect of 5-HTP on stress-induced eating: mean ± S.E.M. food intake in rats administered intraperitoneally with various doses of 5-HTP then allowed access to preweighed chow pellets while they were receiving tail pinches * $P<.05$, when compared to the vehicle-treated group (control) as determined by ANOVA and post hoc Students–Newman–Keuls test (treatment group ns = 6–8).

3.1.4. 5-HTP and other LNAA in plasma and brain

Plasma and brain concentrations of 5-HTP and other LNAA were measured in rats given 5-HTP (0, 50, 100, and 200 mg/kg ip) and sacrificed 1 h later, i.e., the time that animals in the above experiments had been presented with food. Dose-dependent increases were observed in both plasma and brain 5-HTP levels. Plasma levels rose from 28.8 ± 10.8 to 64.2 ± 7.9, 155.3 ± 16.2, and 348 ± 58.4 μM after the 50-, 100-, and 200-mg/kg doses, respectively.

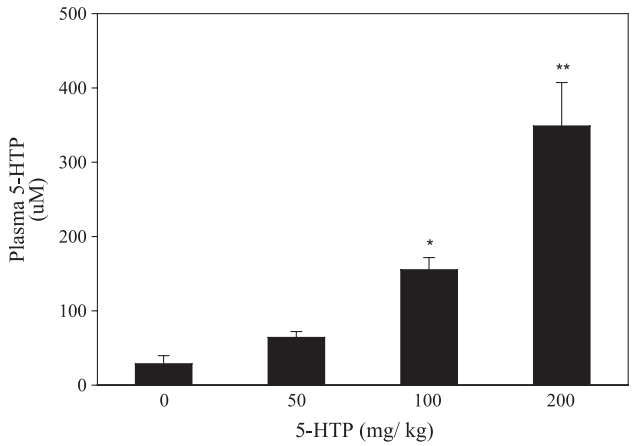

Fig. 4. 5-HTP in plasma 1 h after 5-HTP injection: mean ± S.E.M. plasma 5-HTP in 4-h fasted rats intraperitoneally administered with various doses of 5-HTP. * $P<.05$, ** $P<.001$, when compared to the vehicle-treated group (control) as determined by ANOVA and post hoc Students–Newman–Keuls test (group ns = 6).

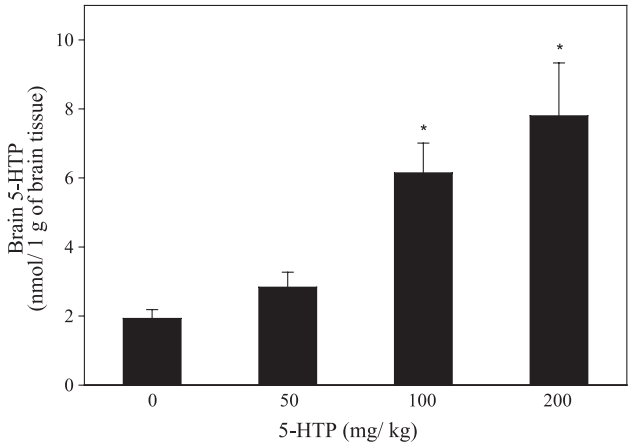

Fig. 5. 5-HTP in brain 1 h after 5-HTP injection: mean ± S.E.M. brain 5-HTP in 4-h fasted rats intraperitoneally administered with various doses of 5-HTP. * $P<.05$, when compared to the vehicle-treated group (control) as determined by ANOVA and post hoc Students–Newman–Keuls test (group $ns=6$).

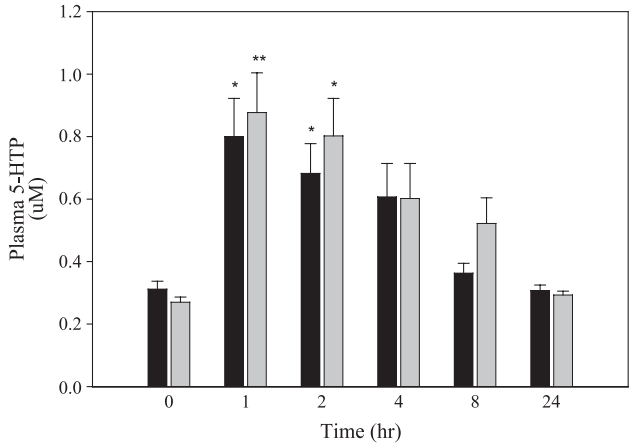

Fig. 7. Plasma 5-HTP at intervals after subjects received 5-HTP: mean ± S.E.M. plasma 5-HTP in healthy human subjects administered 5-HTP 100 ■ or 150 mg po . * $P<.05$, ** $P<.001$, when compared to the corresponding control value at time 0 (baseline) as determined by ANOVA with repeated measures and post hoc Students–Newman–Keuls test (group $ns=6$).

These increases were statistically significant (Fig. 4); the ANOVA yielded $F(3,20)=21.31$, $P<.001$. When compared to vehicle control condition, both 100- and 200-mg/kg doses of 5-HTP significantly increased plasma level of the drug ($P<.05$ or .001) as determined by Newman–Keuls test. The 50-mg/kg dose failed to reach statistical significance ($P>.05$). Brain 5-HTP levels were also increased from 1.9 ± 0.3 to 2.8 ± 0.4, 6.2 ± 0.9, and 7.8 ± 1.5 nmol/g, after the 50-, 100-, and 200-mg/kg doses, respectively (Fig. 5); the ANOVA yielded $F(3,20)=9.12$, $P<.001$. When compared to vehicle control condition, both 100- and 200-mg/kg doses of 5-HTP significantly increased brain level of the drug ($P<.05$) as determined by Newman–Keuls test.

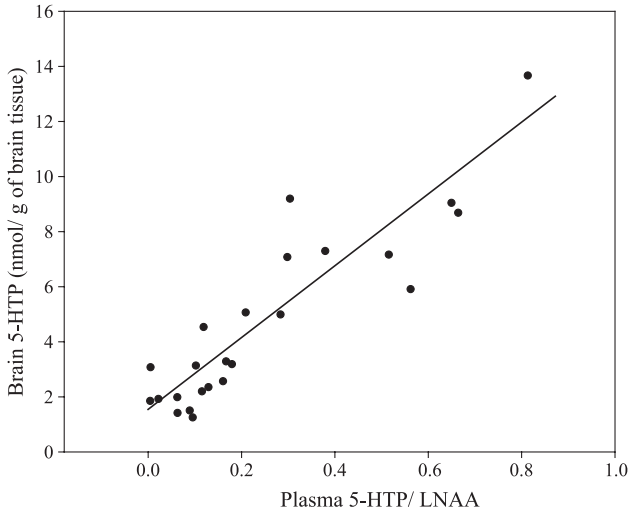

Fig. 6. Correlation between the plasma 5-HTP/LNAA ratio and brain 5-HTP levels in individual rats. The plasma 5-HTP/LNAA ratio was calculated for each rat of the four 5-HTP treatment groups (0, 50, 100, and 200 mg/kg ip) and was plotted against the 5-HTP level in brain. The relation was found to be linear with $r^2=.81$, $P<.001$.

Plasma levels of the other major LNAA (tryptophan, phenylalanine, tyrosine, leucine, isoleucine, methionine, and valine) were also measured, and the plasma 5-HTP/LNAA ratios calculated for each rat in the four 5-HTP treatment groups (0, 50, 100, and 200 mg/kg). That ratio was plotted against brain 5-HTP levels and exhibited a significant linear relationship with a correlation coefficient of $r^2=.8098$, $F(1,22)=93.68$, $P<.001$ (Fig. 6).

3.2. Human studies

3.2.1. Effects of 5-HTP on plasma 5-HTP levels in humans

Administration of 100 or 150 mg 5-HTP orally to healthy human volunteers produced significant dose-related increases in the plasma 5-HTP levels after 1 and 2 h (Fig. 7); the ANOVAs yielded $F(5,30)=7.22$ and 7.58 (all $P<.001$). Basal levels of 0.31 ± 0.02 μM were significantly increased to 0.80 ± 0.12 and 0.68 ± 0.09 μM 1 and 2 h after the 100-mg dose ($P<.05$ when compared to basal levels as determined by Newman–Keuls test), and to 0.88 ± 0.13 and 0.80 ± 0.12 μM 1 and 2 h after the 150-mg dose ($P<.05$ or .001 when compared to basal levels as determined by Newman–Keuls test). Analysis of the area under the plasma concentration–time curve demonstrated a significant increase (data not shown).

4. Discussion

These data show that 5-HTP, injected peripherally into rats without a decarboxylase inhibitor, produces dose-related increases in plasma and brain 5-HTP levels and also suppresses eating, particularly that generated by an experimental stressor, tail pinch. Moreover, oral 5-HTP given without a decarboxylase inhibitor also produces similar

increases in plasma 5-HTP in humans. Hence, it may be possible to use relatively low doses of 5-HTP to suppress excessive food consumption in stressed individuals or modify other serotonin-dependent behaviors, without also administering a decarboxylase-inhibiting drug.

The entry of plasma 5-HTP into the brain is competitive with L-valine (Fig. 4) and thus presumably with other LNAA (Fernstrom et al., 1979; Maher et al., 1984; Pardridge, 1998). Hence, it might be expected that the ability of a 5-HTP dose to enhance serotonin-mediated behaviors would be increased by dietary carbohydrates (which decrease plasma LNAA) and diminished by dietary proteins (which increase plasma LNAA) (Fernstrom and Wurtman, 1971). Similar interactions with foods that affect plasma LNAA levels have been demonstrated for L-DOPA, a related, therapeutically useful amino acid (Berry et al., 1991).

Large doses of 5-HTP have previously been shown to cause short-term decreases in food intake (Ceci et al., 1989), particularly of carbohydrate-rich foods (Cangiano et al., 1992, 1998). The involvement of brain serotonin in the mechanisms controlling macronutrient intake and the ability of serotonin agonists to suppress carbohydrate intake have been demonstrated repeatedly (Wurtman and Wurtman, 1979). This relationship underlies the use of serotoninergic drugs (or dietary carbohydrates, which raise brain serotonin levels; Fernstrom and Wurtman, 1972) to treat clinical conditions associated with carbohydrate craving, e.g., obesity (Wurtman et al., 1979), premenstrual syndrome (Brzezinski et al., 1990), smoking withdrawal (Spring et al., 1991), or seasonal affective disorder (O'Rourke et al., 1989). Giving carbohydrates with 5-HTP might be expected to augment the anorexic effects of both.

Administration of 5-HTP could cause at least transient increases in plasma serotonin levels. Such serotonin would quickly be removed from the plasma by uptake into platelets or by the activity of hepatic or renal monoamine oxidase (Maher et al., 1999). If the 5-HTP were also given with drugs that blocked both of these processes, larger and more prolonged increases in plasma serotonin might occur, conceivably generating clinical findings similar to those seen in the carcinoid syndrome (Robiolio et al., 1995). Such findings have been observed in patients who received fenfluramine, an inhibitor of platelet serotonin uptake, along with phentermine, an monoamine oxidase inhibitor (Maher et al., 1999; Ulus et al., 2000).

The greatly enhanced sensitivity of stressed rats to the anorexic effect of 5-HTP (Fig. 5) provides additional evidence that some stress effects can be ameliorated by treatments that enhance serotonin-mediated neurotransmission (Clark et al., 1992; Graeff et al., 1996). The role of stress-activated mechanisms in the architecture of feeding behaviors has been recently recognized as a potentially important site for therapeutic manipulation (Halford and Blundell, 2000). Since oral 5-HTP given without a decarboxylase inhibitor elevates plasma 5-HTP levels in humans, it is conceivable that this amino acid might be useful in diminishing the overeating and perhaps other symptoms sometimes associated with stress.

Acknowledgements

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