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Effect of Chronic Corn Consumption on Serotonin Content of Rat Brain

SEROTONIN, a putative neurotransmitter in the mammalian central nervous system, is synthesized in the brain by the 5-hydroxylation and decarboxylation of the essential amino-acid L-tryptophan[1,2]. The control of serotonin biosynthesis seems to involve a different mechanism from that responsible for catecholamine biosynthesis[3,4] in its dependence on the availability of the amino-acid precursor[5,6]. Thus, small doses of tryptophan that do not increase brain or plasma tryptophan concentrations beyond their normal daily ranges cause significant increases in the serotonin concentration of rat brain[7]. Conversely, the chronic ingestion of diets lacking in tryptophan (with casein hydrolysates or amino-acid mixtures substituted for natural proteins) depresses brain serotonin levels[8–10]. The dependence of serotonin biosynthesis on tryptophan availability probably arises from the unusually high substrate K_M that characterizes tryptophan hydroxylase[1]. It seems likely that this enzyme normally functions in an unsaturated state; hence physiological increases in intraneuronal tryptophan could drive the hydroxylation of the amino-acid and, ultimately, its conversion to serotonin.

Mammals are unable to synthesize tryptophan, so that all of the tryptophan available for conversion to serotonin in their brains must ultimately be derived from dietary proteins. The proportion of tryptophan within most such proteins is usually very low[11]; in corn protein, the concentrations of tryptophan and lysine are so low that they may limit the ability of the body to utilize the other amino-acids for protein synthesis[12]. To determine whether the tryptophan deprivation resulting from the chronic ingestion of a naturally occurring tryptophan-poor protein can also interfere with serotonin biosynthesis, we examined brain serotonin levels in young adult rats given a diet with corn as the sole protein source. Our results indicate that

Table 1 Composition of Experimental Diets

Ingredient	Diet* 18% casein	6% casein	Corn
Casein	180	60	—
Corn	—	—	800
Dextrose	207	250	—
Sucrose	167	201	—
Dextrin	207	250	—
Mazola oil	150	150	150
Harper's salt[26]	40	40	40
Vitamin mix[27]	10	10	10
Choline (50%)	2 ml.	2 ml.	2 ml.
Agar	35	35	—
Water	1,000	1,000	1,000

*g/2 kg of diet wet weight.

corn consumption is associated with a marked reduction in brain serotonin and that this reduction can be largely corrected by adding tryptophan to the diet.

Groups of fifteen male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts) initially weighing 53–84 g consumed one of the following diets for 5 weeks (Tables 1 and 2). (1) Corn diet (containing 80% dry enriched white Masa Harina corn, given by Quaker Oats Co., Corn Products Division, Chicago; the corn contains 7% protein), with *ad libitum* access; (2) corn diet supplemented with 0.1% L-tryptophan, pair-fed with group 1; (3) 6% casein diet (a control diet in which the protein content was similar to that of the corn diet), pair-fed with group 1; (4) 6% casein diet, with *ad libitum* access; (5) 18% casein diet (a diet containing a protein concentration similar to that present in most commercial rat chows), also with *ad libitum* access. The animals were decapitated between 1500 and 1800 h, 6–9 h after the onset of the daily light period. Their brains were frozen on dry ice; blood from the cervical wound was collected in heparinized tubes, centrifuged, and its plasma frozen until assayed. The tryptophan in plasma and brain was assayed by the fluorimetric method of Denckla and Dewey[13]; brain serotonin was assayed by the method of Thompson *et al.*[14].

The animals eating the 18% casein diet (group 5) consumed about 50% more food each day than rats in any of the other groups (Table 2). The failure of the rats in group 4 to consume more of the 6% casein diet than those in group 3 is compatible with other observations[15] that rats choose to eat less of a diet if it contains an inadequate fraction of protein. The total

amount of tryptophan ingested daily by each group varied from 2.9 mg in the animals fed corn (group 1) to 22.1 mg in the animals given the 18% casein diet (Table 2). Plasma tryptophan concentrations reflected these differences in tryptophan consumption; rats in group 1 had the lowest concentrations, while those of animals eating corn fortified with tryptophan (group 2) were almost twice as great (Table 2). Like plasma tryptophan, brain tryptophan concentrations were lowest among rats in group 1 and highest in animals eating the 18% casein diet (Table 2); those of the rats in the other three groups were intermediate and did not differ significantly from each other.

The concentrations and absolute amounts of serotonin in the brains of rats fed the corn diet were, respectively, 28% and 38% lower than in brains of animals consuming the 18% casein diet (Table 2). Fortification of the corn with tryptophan (group 2) significantly raised brain serotonin content above that of rats in group 1, and raised brain serotonin concentrations to values approaching those of group 5 (Table 2). The serotonin levels and concentrations of rats consuming the 6% casein diet (groups 3 and 4) were only slightly higher ($P<0.05$, comparing total contents) than those of the rats fed corn. All of the animals consuming diets containing 5.4–5.7% protein grew less well than group 5 (Table 2). Body weight increased slightly more rapidly in groups 3 and 4 than in groups 1 and 2, possibly because the casein diet provided more lysine[11]. The brain weights of rats in groups 1–4 did not differ significantly from each other, and were about 11% lower than those of the animals in group 5 (Table 2).

The concentrations of tryptophan in rat plasma[16] and brain[6,7] and of serotonin in rat brain[17] exhibit characteristic daily fluctuations. To rule out the possibility that the apparent effects of eating corn were simply a result of the particular time of day at which our animals were killed, additional rats, treated similarly to groups 1 and 5, were killed at 6 h intervals. The animal room was lighted between 0900 and 2100 h; groups were killed at 0100, 0700, 1300 and 1900 h. Brain serotonin concentrations were significantly lower ($P<0.01$) in corn-fed rats at all times tested; they were most depressed, compared with those of animals fed casein, when measured at 0100 and 0700 h (57% and 67% of control). Plasma tryptophan was significantly depressed ($P<0.001$) at all times studied; brain tryptophan concentrations were depressed in corn-fed animals at 0700, 1300 and 1900 h ($P<0.01$) but not at 0100 h.

These data show that the concentration of serotonin in the brain of the post-weanling rat is significantly depressed when the animal is given access *ad libitum* to a tryptophan-poor corn diet. The hypothesis that inadequate tryptophan caused this

Table 2 Effects of Various Diets on Tryptophan Intake and Brain Serotonin Levels

	Treatment group				
	1	2	3	4	5
Protein source	Corn	Corn+ tryptophan (0.1%)	Casein	Casein	Casein
Access to diet	*ad lib.*	Pair-fed with *1*	Pair-fed with *1*	*ad lib.*	*ad lib.*
Protein content of diet (% dry weight)	5.6	5.7	5.4	5.4	16.2
Tryptophan content of diet (mg/100 g dry weight)	32	132	55	55	164
Average daily food consumption (g/day, dry weight)	9	8.5	7.5	8	13.5
Average daily tryptophan consumption (mg/day)	2.9	11.2	4.1	4.4	22.1
Body weight (g)					
Initial	58	67	64	70	68
Final	82	95	98	108	251
% increment	41	42	53	54	269
Brain weight (g)	1.65±0.01	1.69±0.02	1.70±0.02	1.74±0.02	1.92±0.01*
Plasma tryptophan (μg/ml.)	3.31±0.22	6.17±0.52*	5.15±0.31 *	4.51±0.46*	12.26±0.60*
Brain tryptophan (μg/g)	4.09±0.41	6.98±0.48*	6.97±0.36*	8.89±0.21*	10.50±0.17*
Brain serotonin (μg/g)	0.47±0.02	0.60±0.01*	0.51±0.02	0.50±0.02	0.65±0.01*
(μg/brain)	0.78±0.04	1.01±0.03*	0.86±0.03	0.87±0.03	1.25±0.03*

*$P<0.001$ compared with corn group (1).

reduction is supported by the finding that the addition of tryptophan to the corn diet greatly increased brain tryptophan levels, and raised brain serotonin concentrations almost to normal (Table 2). These results also demonstrate that chronic consumption of a low-protein diet, even with proportionately adequate amounts of tryptophan (the 6% casein diet), can also lower brain tryptophan and serotonin concentrations. Animals given this diet consume much less food than rats fed diets containing adequate protein (for example, 18% casein), and thereby decrease their total tryptophan intake. Apparently, brain serotonin levels can be depressed to varying degrees by a variety of nutritional manipulations in which low-protein food sources are provided.

The correlations between plasma and brain tryptophan and brain serotonin concentrations among the various experimental groups were not perfect; rats in group 2 (corn fortified with tryptophan) had higher plasma tryptophan and brain serotonin concentrations than animals in group 4, but lower brain tryptophan concentrations (Table 2). Several factors may be responsible for this apparent discrepancy. First, we measured total plasma tryptophan and not free tryptophan[18]. As it is possible that only the free tryptophan pool is in equilibrium with brain tryptophan, our data can provide only an indication of the true plasma tryptophan concentration exchanging with the amino-acid in the brain. Second, the tryptophan concentrations in homogenates of whole brain may be only suggestive of the concentrations within those relatively few brain cells that convert tryptophan to serotonin. Third, tryptophan and serotonin levels were measured in the same brains; thus alterations in serotonin that might have occurred 1 or 2 h after changes in tryptophan could be missed. (We have shown that the increase in brain serotonin that follows tryptophan administration requires at least 60 min to become apparent[7].) Finally, there is no reason to believe that the relationships between plasma and brain tryptophan concentrations, or between brain tryptophan levels and brain serotonin synthesis, are necessarily linear throughout their dynamic ranges.

If the depressed brain serotonin levels observed in rats fed corn reflect impaired serotonin synthesis, our data suggest that when tryptophan is in short supply, the body is not able fully to compensate by preferentially shunting the amino-acid into indoleamine synthesis. Studies in our laboratory[19] have shown that severe protein malnutrition suppresses the rise in brain catecholamine levels normally observed in weanling rats; however, brain catecholamine levels do not fall in adult rats after 10 days (Zigmond and Shoemaker, unpublished observations) or 16 days[20] of phenylalanine and tyrosine deprivation.

If brain serotonin levels are indicative of the physiological

activity of serotonin-containing neurones, our study raises the possibility that certain brain functions might be significantly impaired when dietary tryptophan is chronically inadequate. Some specific functions that may be associated with serotonin-containing neurones include the control of sleep[21], mood or affect[22], body temperature[23], and the secretion from the anterior pituitary gland[24]. The changes in brain serotonin induced by the corn diet occurred in the presence of continued (if attenuated) body growth, and were associated with only relatively small changes in brain growth (Table 2). In contrast, the low brain catecholamine levels found in weanling rats subjected to perinatal protein undernutrition[19,25] have been observed only in grossly malnourished animals with greatly reduced body and brain weight. In certain societies, large numbers of people subsist on corn diets as their major protein source. These people may have short stature and low body weight, but often do not present striking clinical evidence of overt malnutrition. Our findings on rats raise the possibility that such diets might modify the functional activity of an important set of brain neurones in these people.

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[1] Lovenberg, W., Jequier, E., and Sjoerdsma, A., *Adv. Pharmacol.*, **6A**, 21 (1968).
[2] Lovenberg, W., Weissbach, H., and Udenfriend, S., *J. Biol. Chem.*, **237**, 89 (1962).
[3] Nagatsu, T., Levitt, M., and Udenfriend, S., *J. Biol. Chem.*, **239**, 2910 (1964).
[4] Weiner, N., *Ann. Rev. Pharmacol.*, **10**, 273 (1970).
[5] Moir, A. T. B., and Eccleston, D., *J. Neurochem.*, **15**, 1093 (1968).
[6] Wurtman, R. J., and Fernstrom, J. D., in *Perspectives in Neuropharmacology* (edit. by Snyder, S. H.) (Oxford University Press, New York, in the press).
[7] Fernstrom, J. D., and Wurtman, R. J., *Science*, **173**, 149 (1971).
[8] Zbinden, G., Pletscher, A., and Studer, A., *Z. Ges. Exp. Med.*, **129**, 615 (1958).
[9] Gal, E. M., and Drewes, P. A., *Proc. Soc. Exp. Biol. Med.*, **110**, 368 (1962).
[10] Culley, W. J., Saunders, R. N., Mertz, E. T., and Jolly, D. H., *Proc. Soc. Exp. Biol. Med.*, **113**, 645 (1963).
[11] Block, R. J., and Weiss, K. W., *Amino Acid Handbook*, 249 (Charles C. Thomas, Springfield, Illinois, 1956).

[12] Bressani, R., Elias, L. G., and Gomez-Brenes, R. A., *J. Nutrit.*, **97,** 173 (1969).
[13] Denckla, W. D., and Dewey, H. K., *J. Lab. Clin. Med.*, **69,** 160 (1967).
[14] Thompson, J. H., Spezia, C. A., and Angulo, M., *Experientia*, **26,** 327 (1970).
[15] Harper, A. E., in *Mammalian Protein Metabolism* (edit. by Munro, H. N., and Allison, J. B.), **2,** 87 (Academic Press, New York, 1964).
[16] Fernstrom, J. D., Larin, F., and Wurtman, R. J., *Life Sci.*, **10,** 813 (1971).
[17] Dixit, B. N., and Buckley, J. P., *Life Sci.*, **6,** 755 (1967).
[18] McMenamy, R. H., and Oncley, J. L., *J. Biol. Chem.*, **233,** 1436 (1958).
[19] Shoemaker, W. J., and Wurtman, R. J., *Science*, **171,** 1017 (1971).
[20] Green, H., Greenberg, S. M., Erickson, R. W., Sawyer, J. L., and Ellison, T., *J. Pharmacol. Exp. Therap.*, **136,** 174 (1962).
[21] Hery, F., Pujol, J.-F., Lopez, M., Macon, J., and Glowinski, J., *Brain Res.*, **21,** 391 (1970).
[22] Bowers, jun., M. B., *Neuropharmacology*, **9,** 509 (1970).
[23] Beckman, A. L., and Eisenman, J. S., *Science*, **170,** 334 (1970).
[24] *Brain Monoamines and Endocrine Function* (edit. by Wurtman, R. J.), Neurosciences Research Program Bulletin, **9,** No. 2 (1971).
[25] Sereni, F., Principi, N., Perletti, L., and Sereini, L. P., *Biol. Neonat.*, **10,** 254 (1966).
[26] Rogers, Q., and Harper, A. E., *J. Nutrit.* **87,** 267 (1965).
[27] Wurtman, R. J., Shoemaker, W. J., and Larin, F., *Proc. US Nat. Acad. Sci.*, **59,** 800 (1958).

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