Effects of Phenylalanine, Phenylacetic Acid, Tyrosine, and Valine on Brain and Liver Serotonin in Rats. (28675)

DALLAS E. BOGGS, ROBERT ROSENBERG AND HARRY A. WAISMAN
Joseph P. Kennedy, Jr. Memorial Laboratory, Dept. of Pediatrics, Univ. of Wisconsin, Madison

The decrease in brain 5-hydroxytryptamine (serotonin) in rats made phenylketonuric by excess dietary phenylalanine has been well documented(1). It would be of interest to know whether phenylalanine *per se* or its metabolites are responsible for this change. Such information may provide clues as to the mechanism of the so-called "indole defect" in phenylketonuria. Several possibilities to explain this alteration in tryptophan metabolism include: 1) inhibition of the enzyme, 5-hydroxytryptophan (5-HTP) decarboxylase, responsible for formation of serotonin from 5-HTP(2), 2) inhibition of the transport of 5-HTP across the blood-brain-barrier (3), 3) inhibition of the formation of 5-HTP in the liver or other tissues(4), 4) and the frequently neglected possibilities of decreased absorption of tryptophan from the intestinal tract as well as defective reabsorption of this amino acid from the renal tubules(5). As part of the attempt to get proper data on this question it seemed advisable to begin by feeding tyrosine as an amino acid closely related to phenylalanine and by providing phenylacetic acid as an end metabolite of phenylalanine. The effect of valine was studied because it was an amino acid not metabolically related to phenylalanine.

This report will provide data from controlled experiments designed to study the influence of these factors on serotonin content of brain and liver.

Experimental. Eighteen-day old male albino rats (Holtzman Rat Co.) were housed in individual cages in a dark-light room as previously described(6) and provided with their respective diets consisting of a ground commercial rat ration (Rockland) to which was added 3-9% of L-tyrosine, L-phenylalanine, phenylacetic acid, or L-valine. Control rats were fed the same ration, either *ad lib* or paired-fed by providing the control rats with the amount consumed by the respective experimental groups. After 1, 2, or 3 weeks on the diets, the animals were sacrificed by decapitation and the brain serotonin determined on the fresh brains and liver by the method of Bogdanski, *et al*(7).

Results. Table I shows the effects of 3 and 5% phenylacetic acid, 7% L-tyrosine, and 6% L-valine on brain and liver serotonin of rats on the respective diets for 1, 2, and 3 weeks. Phenylacetic acid caused an increase in brain serotonin and a decrease in liver serotonin. Tyrosine had an insignificant effect on brain and some decrease in liver serotonin, whereas 6% valine decreased brain serotonin but not liver serotonin. Table II compares serotonin levels of rats given 7% L-tyrosine, 5% phenylacetic acid, and 6% and 9% L-valine to serotonin levels of rats receiving 5 and 7% L-phenylalanine. The control animals were paired-fed in this series. In contrast to the *ad lib* controls in Table I, the paired-fed controls in Table II show decreased liver serotonin comparable to the levels in rats receiving the respective experimental diets. In other words, paired-feeding decreased liver serotonin without any significant effect on brain serotonin. The brain serotonin levels of the experimental animals are changed in the same direction as the *ad lib* controls. Of all the compounds fed, phenylalanine caused the greatest effect on brain serotonin whether fed at the 5% or 7% level.

Discussion. Considering the close structural similarity between tyrosine and phenylalanine, it is surprising that phenylalanine has a greater influence on serotonin content of brain than tyrosine, phenylacetic acid or valine. The report that phenylacetic acid is an effective inhibitor of 5-HTP decarboxylase, would lead one to expect that this compound would interfere with the formation of brain serotonin. The increase in brain serotonin was therefore unanticipated, but a number of factors regulate the serotonin content in tissues, namely those involved in storage, synthesis, and degradation. Because of these

TABLE I. Effect of Phenylacetic Acid, Tyrosine, and Valine on Brain and Liver Serotonin in the Rat.

Diet	Duration, wk	Serotonin, μg/g wet tissue: Brain Exp	Brain Control‡	Liver Exp	Liver Control‡
3% Phenylacetic acid	1 (4, 6)§	.47 ± .02*	.42 ± .05	.21 ± .07†	.33 ± .05
	2 (5, 6)	.54 ± .03†	.40 ± .09	.40 ± .09	.42 ± .05
	3 (5, 5)	.60 ± .09*	.47 ± .04	.36 ± .13*	.53 ± .07
5% " "	1 (6, 5)	.53 ± .04†	.43 ± .04	.21 ± .02†	.44 ± .12
	2 (6, 5)	.60 ± .05†	.43 ± .02	.20 ± .08†	.44 ± .01
	3 (6, 6)	.67 ± .05†	.54 ± .06	.31 ± .13*	.58 ± .14
7% L-Tyrosine	1 (6, 6)	.43 ± .06	.37 ± .10	.34 ± .03	.42 ± .08
	2 (6, 6)	.47 ± .06*	.54 ± .06	.35 ± .07†	.63 ± .13
	3 (6, 6)	.53 ± .06	.57 ± .07	.41 ± .09*	.59 ± .05
6% L-Valine	1 (6, 6)	.45 ± .03*	.49 ± .03	.41 ± .01	.43 ± .03
	2 (6, 6)	.49 ± .07*	.59 ± .07	.43 ± .07	.43 ± .05
	3 (6, 6)	.59 ± .11†	.86 ± .12	.56 ± .16	.52 ± .08

* Difference significant at $P < .05$.
† Difference significant at $P < .01$.
‡ All control animals were fed *ad lib* except the valine controls, which were pair-fed.
§ Numbers in parentheses represent No. of animals for group, experimental and control groups respectively.

TABLE II. Relative Effects of Phenylalanine, Tyrosine, Phenylacetic Acid, and Valine on Brain and Liver Serotonin in Rats.

Diet‡	Serotonin, μg/g wet tissue: Brain Exp	Brain Paired control	Brain % difference	Liver Exp	Liver Paired control
5% L-Phenylalanine (6, 6)§	.42 ± .16	.68 ± .10	−32.2†	.46 ± .11	.42 ± .08
7% " (6, 6)	.23 ± .07	.48 ± .09	−45.0†	.21 ± .04	.28 ± .03
7% L-Tyrosine (4, 4)	.51 ± .07	.53 ± .06	− 3.7	.40 ± .06	.35 ± .05
5% Phenylacetic acid (6, 6)	.80 ± .15	.65 ± .10	+22.3*	.23 ± .04	.29 ± .08
6% L-Valine (6, 6)	.49 ± .07	.59 ± .07	−17.0*	.43 ± .07	.43 ± .05
9% " (6, 6)	.39 ± .03	.48 ± .10	−17.9†	.27 ± .05	.27 ± .06

* Significant at $P < .05$.
† Significant at $P < .01$.
‡ All animals were on diet for 2 wk, and control animals were pair-fed.
§ Numbers in parentheses represent No. of animals per group, experimental and control groups respectively.

circumstances an isolated study designed to measure any of these factors individually, cannot reliably predict the influence of a chemical substance on tissue serotonin. For example, one chemical may interfere with synthesis, and at the same time may block degradation or cause release from the storage site.

The data in Table II indicate that the degree of change in brain serotonin resulting from excess phenylalanine was greater than that resulting from any of the other substances studied. This cannot be explained in terms of the effect of these compounds on 5-HTP decarboxylase as demonstrated by *in vitro* studies since phenylacetic acid was a better inhibitor than phenylalanine(2). The effect of the amino acids on brain serotonin content likewise cannot be explained by studies of transport of 5-HTP across the blood-brain-barrier as reported by McKean *et al*(3) who showed no dramatic differences between phenylalanine and tyrosine or other L-amino acids studied. These workers further reported that L-tryptophan decreased uptake of 5-HTP by the brain (though this may have been a result of isotopic dilution) in contrast to reports that dietary tryptophan

actually increases brain serotonin(1). Therefore from such studies it is not possible to predict the net effect of a compound on brain serotonin.

The dramatic effects of phenylalanine on brain serotonin could be explained by a combination of factors already known to affect serotonin (or indole) metabolism. However, is is likely that phenylalanine also influences the absorption of tryptophan, the dietary precursor of 5-HTP and serotonin, from the intestinal tract. This approach is particularly worthy of attention since unabsorbed tryptophan is known to be converted by the intestinal bacteria into indole acetic and indole lactic acids which are absorbed and re-excreted in the urine as dramatized in the malabsorption syndrome known as Hartnup's disease(8). Such an effect could very well provide an explanation why phenylketonuric children excrete increased indole acetic and indole lactic acids(9). The probable effects of excess phenylalanine or other amino acids on renal re-absorption of tryptophan could further complicate this picture.

The self imposed food deprivation resulting from the excess amino acids in the diet caused decreased liver serotonin by a mechanism which is not yet understood. Likewise, it is not easy to explain why the brain, and not the liver, of animals on restricted food intake was able to accumulate normal amounts of serotonin. Evidently the brain is more resistant to the result of semi-starvation than the liver and is therefore a better indicator for interpreting the effects of amino acids.

The decreased food intake in the control animals which were paired-fed to those receiving 5% phenylacetic acid accounted for a slight but insignificant increase in brain serotonin, but phenylacetic acid itself caused a significant increase in brain serotonin. Culley *et al*(10) found no effect of feeding 4% phenylacetic acid, but they used older animals than were used in our studies. Therefore, this percentage may not have been sufficiently high in the older animals to cause a change.

The data demonstrate that 6% and 9% L-valine bring about a decrease in brain serotonin. Although valine is structurally unrelated to the aromatic amino acids, it can cause a decrease in brain serotonin. It may well be that other unrelated amino acids can also influence serotonin metabolism, but valine administration serves to illustrate the problem of interdependence of amino acid metabolism in the brain. The role of other amino acids influencing membrane transport and other factors regulating brain serotonin content is worthy of further study.

Summary. A study of the differential effects of excess dietary L-phenylalanine, L-tyrosine, L-valine, and phenylacetic acid on serotonin content of rat brain and liver indicated that phenylalanine is the most effective of these compounds. Five and 7% L-phenylalanine caused a decrease mainly in the brain. Seven percent tyrosine caused little or no change. Brain serotonin decreased when 6% L-valine was fed and brain and liver serotonin decreased when valine was included as 9% of the diet. Most of the change in liver serotonin is due to decreased food intake, since paired-fed control rats also showed decreased serotonin in this tissue. Phenylacetic acid caused an increase in brain serotonin, an effect opposite to that of phenylalanine or valine.

The valuable technical assistance of Daren McLay and Robert Colwell is acknowledged.

1. Wang, H. L., Harwalker, V. H., Waisman, H. A., *Arch. Biochem. Biophys.*, 1962, v96, 181.

2. Huang, I., Hsia, D. Y.-Y., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 81.

3. McKean, C. M., Schanberg, S. M., Giarman, N. J., *Science,* 1962, v137, 604.

4. Freedland, R. A., Wadzinski, I. M., Waisman, H. A., *Biochem. Biophys. Res. Comm.,* 1961, v6, 227.

5. Webber, W. A., *Am. J. Physiol.* 1962, v202, 577.

6. Wang, H. L., Waisman, H. A., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 332.

7. Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *J. Pharm. Exp. Ther.,* 1956, v117, 82.

8. Halvorsen, K., Halvorsen, S., *Pediatrics,* 1963, v31, 29.

9. Pare, C. M. B., Sandler, M., Stacey, R. S., *Lancet,* 1957, v272, 1, 551.

10. Culley, W. J., Saunders, R. N., Mertz, E. T., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 444.

Received July 15, 1963. P.S.E.B.M., 1963, v114.