Influence of Monoamine Oxidase Inhibitors on 5-Hydroxytryptamine Synthesis in the Brain.* (31395)

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Tryptophan and 5-hydroxytryptophan injected intraperitoneally into rats will cause an increase in the 5-hydroxytryptamine content of brain(1-4). This increase is accentuated when monoamine oxidase is inhibited. The combination of either precursor of 5-hydroxytryptamine and monoamine oxidase inhibition causes dramatic behavioral changes characterized by front paw clonus, piloerection, exophthalmia and body tremors.

Monoamine oxidase inhibitors are used frequently to study central nervous system increases of 5-hydroxytryptamine. The purpose of this investigation was to study the effects of some monoamine oxidase inhibitors on the conversion of tryptophan and 5-hydroxytryptophan to 5-hydroxytryptamine. Dubnick *et al*(5) have demonstrated that the potent monoamine oxidase inhibitor β-phenylethylhydrazine can inhibit 5-hydroxytryptophan decarboxylase *in vitro* and *in vivo*. Gal *et al* (6) have demonstrated that in β-phenylisopropylhydrazine-treated animals the conversion of tryptophan to 5-hydroxytryptophan is significantly less. In this experiment an attempt is made to compare the behavioral effects observed from the combination of inhibitor and precursor to levels of 5-hydroxytryptamine and to the per cent of monoamine oxidase inhibition.

Two hydrazine and two non-hydrazine inhibitors are utilized in this investigation. Iproniazid (1-isonicotinyl-2-isopropylhydrazine) and β-phenylisopropylhydrazine are classical hydrazine inhibitors of monoamine oxidase. Green and Sawyer(7) used *trans*-2-phenylcyclopropylamine in studies of 5-hydroxytryptophan conversion to 5-hydroxytryptamine and it is used here as one of the non-hydrazine inhibitors, and the second non-hydrazine compound is pargyline.

Methods. 5-hydroxytryptamine was assayed according to the procedure described by Udenfriend *et al*(8) with the addition of borate buffer washes to remove the 5-hydroxytryptophan.

The method of Kuntzman *et al*(9) was modified for measuring monoamine oxidase activity. A 100-150 mg slice of each brain was homogenized in enough distilled water to make a 3% homogenate. To 1.0 ml of this homogenate was added 0.3 ml of 0.5 M phosphate buffer pH 7.4, 1.2 ml of distilled water and, after 20 minutes incubation in a Dubnoff metabolic shaker, 0.25 M (0.5 ml) 5-hydroxytryptamine was added and incubated for 60 minutes. The disappearance of substrate was calculated by fluorometric determination of the 5-hydroxytryptamine remaining in the incubation medium.

Male Sprague-Dawley rats weighing 160-250 g were used in all the experiments. The monoamine oxidase inhibitors were administered subcutaneously at either 0.5, 8, 16 or 48 hours before L-tryptophan, 204 mg/kg, or DL-5-hydroxytryptophan, 25 mg/kg, was injected intraperitoneally. L-tryptophan treated rats were sacrificed 120 minutes later; DL-5-hydroxytrpytophan animals were sacrificed after 60 minutes. The table in *Results* indicates the combined pretreatment and treatment time.

The behavior effects were subjectively measured on a 0 to 4 scale. The 0 reading represents control, or normal animal behavior. A reading of 1 represents a slight increase in locomotor activity and exploratory activity; 2 represents an intensified increase in locomotor activity and definite signs of piloerection; 3 represents animals showing piloerection, some exophthalmia, intermittent body tremors, aimless wandering, and aggressive behavior to other animals; 4 represents the most severe symptoms, characterized by circling movements, writhing, some loss of postural reflexes, unrested body tremors and front paw clonus, marked piloerection and

* Supported by State of Washington Initiative 171 Funds for Research in Biology and Medicine and by Am. Cancer Soc.

TABLE I. Percent MAO Inhibition.

Inhibitor	Interval after administration 1.5 hr	±SE	9 hr	±SE	17 hr	±SE	49 hr	±SE
Trans-2-phenylcyclopropylamine	98	2	98	1	94	4	75	2
Pargyline	93	6	94	0	89	2	72	2
β-phenylisopropylhydrazine	91	3	85	4	79	5	70	2
Iproniazid	—		96	7	99	1	86	2

n = 3 to 5.

TABLE II. Brain 5-Hydroxytryptamine.

Treatment	1.5 hr μg 5-HT/g	±SE	9 hr μg 5-HT/g	±SE	17 hr μg 5-HT/g	±SE	49 hr μg 5-HT/g	±SE
Control	.56	.02						
TRY*	.73	.02						
5-HTP	.79	.03						
PCP	.78	.04	1.27	.06	1.19	.02	.76	.04
PCP + TRY*	1.34	.14	1.98	.05	1.31	.10	1.06	.07
PCP + 5-HTP	3.50	.31	2.04	.40	1.74	.11	1.04	.05
MO-911	.82	.07	1.15	.05	1.30	.02	.89	.02
MO-911 + TRY*	1.76	.07	1.70	.13	1.61	.06	1.04	.20
MO-911 + 5-HTP	3.23	.28	2.28	.31	2.10	.06	1.20	.07
PIH	.99	.09	1.29	.06	1.20	.04	.92	.05
PIH + TRY*	1.50	.12	1.79	.04	1.59	.07	1.20	.04
PIH + 5-HTP	2.96	.22	2.90	.12	2.39	.18	1.41	.09
IIH			.94	.05	1.13	.06	.85	.02
IIH + TRY*			1.54	.09	1.59	.03	1.23	.07
IIH + 5-HTP			2.90	.08	2.86	.22	1.53	.18

Gives concentration of 5-hydroxytryptamine in μg/g brain, with standard error (SE) of mean. Abbreviations: PIH = β-phenylisopropylhydrazine; IIH = iproniazid; PCP = *trans*-2-phenylcyclopropylamine; MO-911 = pargyline; 5-HT = 5-hydroxytryptamine; 5-HTP = DL 5-hydroxytryptophan; TRY = L-tryptophan.

* Values of 5-HT when TRY is used are determined 1 hr later, *i.e.*, 2.5, 10, 18 and 50 hr.

exophthalmia, and fear type of response to any external stimulus. These ratings were compared to the amount of monoamine oxidase inhibited and to the brain level of 5-hydroxytryptamine. In the text, excitation of the rat is considered any one of the observations 1 to 4.

Results. The percentage of monoamine oxidase inhibition by the 2 hydrazine and 2 non-hydrazine agents is given in Table I. Inhibition with β-phenylisopropylhydrazine and *trans*-2-phenylcyclopropylamine diminishes after 9 hours. The inhibition by pargyline and iproniazid does not change until after 16 hours. The monoamine oxidase inhibitors with L-tryptophan or DL-5-hydoxytryptophan cause excitation, the intensity of which followed the per cent of inhibition. Inhibition by β-phenylisopropylhydrazine and *trans*-2-phenylcyclopropylamine elicited excitation of the rats at 1.5 and 9 hours (3-4) but little at 16 and 48 hours (0-1). In the case of pargyline and iproniazid the excitation is seen at 16 hours (2-4), but does not occur at 48 hours. Iproniazid is the most persistent with a small number of rats becoming excited at 48 hours. Iproniazid, of the 4 agents tested, showed the greatest monoamine oxidase inhibition at 48 hours. The qualitative nature of the measure of the extent of excitation precluded accurate comparison between the various inhibitors. Generally the intensity of excitation was greater (3-4) when monoamine oxidase activity was 15% or less but the intensity of excitation did not appear to be related to the level of 5-hydroxytryptamine.

Table II shows the increase of 5-hydroxytryptamine after the inhibitors plus either L-tryptophan or DL-5-hydroxytryptophan. It is seen that the non-hydrazine inhibitors and

hydrazine inhibitors cause increases in 5-hydroxytryptamine which are very similar. L-tryptophan or DL-5-hydroxytryptophan given after the inhibitors give increases in 5-hydroxytryptamine which are significantly different. The non-hydrazine inhibitors give increases at the first test interval which are greater than those seen with the hydrazine inhibitors. At later intervals increases of 5-hydroxytryptamine by 5-hydroxytryptophan are more nearly the same. There is no significant quantitative difference in the conversion of L-tryptophan to 5-hydroxytryptamine with either hydrazine or non-hydrazine agents.

Discussion. The results indicate that the monoamine oxidase inhibitors used to demonstrate increases of 5-hydroxytryptamine following tryptophan and 5-hydroxytryptophan administration can influence more than degradation of 5-hydroxytryptamine. The non-hydrazine monoamine oxidase inhibitors *trans*-2-phenylcyclopropylamine and pargyline cause a fairly rapid inhibition of monoamine oxidase. During the first few hours of this inhibition 5-hydroxytryptophan gives a substantial increase in 5-hydroxytryptamine. This increase becomes less as the interval after administration lengthens even though monoamine oxidase inhibition is still quite high. Whereas β-phenylisopropylhydrazine is no longer present in tissue in 2 hours(10), *trans*-2-phenylcyclopropylamine is present at least 4 hours (Horita, personal communication). After 9 hours increases of 5-hydroxytryptamine from 5-hydroxytryptophan are the same. It is evident that the initial increased conversion of 5-hydroxytryptophan to 5-hydroxytyptamine is an effect due to the presence of the drug. Dubnick *et al*(11) have shown that β-phenylethylhydrazine, a potent monoamine oxidase inhibitor, causes an increase in 5-hydroxytryptamine formation which cannot be explained in terms of monoamine oxidase inhibition, but occurs in the presence of repeated doses of the drug. Green and Sawyer(7) have indicated that pargyline increases transport of DL-hydroxytryptophan. Another possibility is that decarboxylation could be affected. L-tryptophan is not converted at a significantly higher rate during the first few hours of monoamine oxidase inhibition with the non-hydrazine inhibitors. It would appear that hydroxylation is not affected by the inhibitor. Since hydroxylation is the rate-limiting step, either increased decarboxylation or transport could occur without a change in the amount of 5-hydroxytryptamine formation from L-tryptophan.

In the case of the 2 hydrazine monoamine oxidase inhibitors, iproniazid and β-phenylisopropylhydrazine, there again is a higher conversion of DL-5-hydroxytryptophan to 5-hydroxytryptamine during the early hours after inhibition, but this is much less than in the case of the non-hydrazine inhibitors. If one compares the difference between 5-hydroxytryptamine levels in each monoamine oxidase inhibited group to the levels of 5-hydroxytryptamine after DL-5-hydroxytryptophan it is very evident that the non-hydrazine inhibitors greatly affect the conversion of DL-5-hydroxytryptophan to 5-hydroxytryptamine. In the case of the hydrazine inhibitors, 5-hydroxytryptophan causes increases of 5-hydroxytryptamine which are directly related to the per cent of monoamine oxidase inhibition. With the non-hydrazine inhibitors, 5-hydroxytryptophan causes a much greater increase in 5-hydroxytryptamine than can be explained in terms of monoamine oxidase inhibition. Only after 9 hours is there a correlation between monoamine oxidase inhibition and increase of 5-hydroxytryptamine.

Excitation characterized by front paw clonus, piloerection, exophthalmia and body tremors is marked in rats receiving either the non-hydrazine or hydrazine monoamine oxidase inhibitors and then tryptophan or 5-hydroxytryptophan. An attempt was made to correlate this excitation with levels of 5-hydroxytryptamine but no such correlation was notable. Excitation is always seen during the first and second test periods. At the third test period, 16 hours, all the animals treated with inhibitors show excitation except *trans*-2-phenylcyclopropylamine. At 48 hours, excitation is not observed, with the occasional exception of iproniazid-treated animals. It appears from the data that this excitation is most evident when there exists 85% or more monoamine oxidase inhibition.

The results indicate that although these are long lasting irreversible inhibitors of monoamine oxidase, they can influence precursory steps in the anabolism of 5-hydroxytryptamine. This is especially true for the non-hydrazine inhibitors tested in these experiments. It is also evident from these results that the time period after use of inhibitors is very important—the shorter the time period the greater the chance for interference on systems other than monoamine oxidase.

Summary. Two hydrazine (β-phenylisopropylhydrazine and iproniazid) and 2 non-hydrazine (*trans*-2-phenylcyclopropylamine and pargyline) monoamine oxidase inhibitors were tested for their influence on the conversion of tryptophan and 5-hydroxytryptophan to 5-hydroxytryptamine. It was found that the conversion of 5-hydroxytryptophan was influenced by both types of monoamine oxidase inhibitors but the non-hydrazine inhibitors were most effective. There was an increase in the amount of 5-hydroxytryptamine formed, especially during the first few hours after the inhibitors were administered. Tryptophan conversion to 5-hydroxytryptamine was not significantly influenced by the monoamine oxidase inhibitors. Excitatory responses seen with monoamine oxidase inhibitors and 5-hydroxytryptamine precursors were also observed and appeared to be related to the degree of enzyme inhibition more than to the amount of 5-hydroxytryptamine.

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Received April 4, 1966. P.S.E.B.M., 1966, v123.

Effect of Increased Tissue Traction Upon Tensile Strength of Cutaneous Incisions in Rats.* (31396)

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There are many biological situations in which mechanical factors seem to direct organization and growth of connective tissue. Histological study of tendon indicates that collagen aligns in a direction parallel to the lines of stress, and of bone show that the trabeculae therein align (and are capable of re-aligning) in such a manner as to afford the greatest possible structural stability. The urinary bladder, the uterus and the body's arterioles all become hyperplastic in response to increased pressure on their luminal surfaces. Thus, teleologically speaking, connective tissue is able to organize itself in a manner best suited for function in response to the existing stresses upon that tissue. The response of morphology to stress in the musculoskeletal system has been discussed by Hirsch as the "stress stimuli" principle(5).

The literature has been inconclusive re-

* Supported by the Pangborn Foundation Fund and a grant from Department of the Army, Office of the Surgeon General, 49-193-MD-2450.

† Supported in part by the Charles M. Hitchcock Student Fellowship at University of Maryland School of Med.