In vivo Monoamine Oxidase Inhibition Measured by Potentiation of Tryptamine Convulsions in Rats. (25633)

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Evidence has been presented(1,2) that potentiation of the convulsant action of tryptamine serves as a useful measure of *in vivo* monoamine oxidase (MAO) inhibitory activity. Presumably, the mechanism of potentiation involves prevention of the destruction of tryptamine by MAO. Thus, doses of tryptamine which are sub-convulsant in normal animals because of rapid destruction of tryptamine by MAO, become convulsant in the presence of an MAO inhibitor. Biochemical evidence in support of this concept will be presented (Green and Sawyer, to be published). The present report is concerned with *in vivo* MAO inhibitory activity of a variety of pharmacological agents as measured by potentiation of the convulsant effects of tryptamine.

Methods. The following drugs were investigated: harmine, tranylcypromine (trans-2-phenylcyclopropylamine hydrochloride, SKF *trans* 385); SKF *cis* 385 (*cis*-2-phenylcyclopropylamine hydrochloride); *dl*-PIH (JB-516, α methyl-phenethylhydrazine); *d*-PIH, RO-4-1018 (*dl*-N-acetylmethionyl-isopropylhydrazide); nialamide [N-benzyl-3-(isonicotinylhydrazino) propamide]; phenelzine (β phenethylhydrazine); iproniazid (1-isonicotinyl-2-isopropylhydrazine); isonicotinyl-PIH (1 - isonicotinyl - 2 - phenylisopropylhydrazine dihydrochloride); diphenhydramine hydrochloride; tripelennamine hydrochloride; isoniazid (isonicotinic acid hydrazide); dihydroergotamine; mescaline; SKF 525-A (β-dimethylaminoethyldiphenylpropylacetate hydrochloride); SKF 5-A [2-amino-1-(3,4-methylenedioxyphenyl)-propane hydrochloride]; procaine; and procaine amide. A detailed description of the procedure for measuring potentiation of the convulsant effects of tryptamine has been reported(2) and is only briefly summarized here. Groups of rats were first treated with various doses of the drug under investigation and later, at time of peak drug effect, all rats were injected intravenously with 5 mg/kg of tryptamine hydrochloride. This dose of tryptamine causes convulsions in only 4% of untreated rats and is referred to as a CD_4. The dose of drug effective in causing 50% of rats to respond to the CD_4 of tryptamine with 3 seconds or more of uninterrupted clonic seizure activity (ED_{50}) and 95% fiducial limits was calculated according to the graphic log-probit method of Litchfield and Wilcoxon(3).

Results. A summary of tryptamine potentiating activity of the drugs investigated is presented in Tables I and II. Compounds in Table I effected significant potentiation and therefore permitted ED_{50} determinations. Compounds have been arranged in descending order of potency, subcutaneously administered harmine being the most potent and isonicotinyl-PIH least potent. The compounds in Table II failed to effect significant potentiation of tryptamine's convulsant effects. Diphenhydramine effected a maximum of 43% potentiation at a dose of 150 mg/kg; higher doses caused neurotoxic effects *per se*. None of the remaining compounds listed potentiated tryptamine, in spite of the fact that in most instances barely subtoxic doses of each compound were employed.

Discussion. All of the compounds found to be effective in potentiating the convulsant effects of tryptamine have also been reported to be potent *in vivo* inhibitors of MAO [harmine (4,5,6), tranylcypromine(7,8), SKF *cis* 385 (8), *d*-PIH(9), *dl*-PIH(10), RO-4-1018(11), nialamide(12), phenelzine(13), iproniazid (14) and isonicotinyl-PIH(15). In contrast, many of the compounds found to be devoid of tryptamine potentiating activity [Table II, also see(2)] have also been reported to be devoid of *in vivo* MAO inhibitory activity. It is concluded that the tryptamine potentiation potency of the compounds tested in this series reflects their potency as *in vivo* inhibitors of MAO.

Iproniazid has been used by most investi-

TABLE I. Potencies of Various Compounds as Potentiators of the Convulsant Effects of Tryptamine in Rats.

Drug*	Time of peak effect	ED_{50} (95% fiducial limits), mg/kg	Potency rating (iproniazid = 1)
Harmine (s.c.)	15 min.	.02 (.01- .04)	455
Tranylcypromine	14 hr	.18 (.13- .24)	55
SKF *cis* 385	14 "	.42 (.30- .60)	24
d-PIH	8 "	.56 (.34- .88)	18
dl-PIH	10 "	1.1 (.5 - 2.4)	9
Harmine (oral)	15 min.	4.0 (1.8 - 9.0)	2†
RO-4-1018	18 hr	4.4 (3.0 - 6.4)	2
Nialamide	18 "	5 (3.3 - 7.7)	2
Phenelzine	10 "	8.2 (5.1 -13.3)	1
Iproniazid	18 "	10 (6.3 -16.0)	1
Isonicotinyl-PIH	12 "	18 (14.6 -22.1)	.5

* Harmine was tested both orally and subcut. All remaining compounds were tested orally.

† Not significantly different in potency from iproniazid ($p > .05$).

gators as the standard for comparison of MAO inhibitors. Because of the wide variety of test procedures, species, route of administration, etc., employed by different investigators, it has not been possible to obtain from the literature an accurate comparison of potencies of the various inhibitors. With this aim in mind, relative potencies of effective tryptamine potentiators have been listed in Table I. Orally administered harmine was found to be approximately equivalent in potency to iproniazid, whereas subcutaneously administered harmine was found to be approximately 455 times as potent as iproniazid. This striking difference in potency is in general agreement with the findings of Tabachnick and Rubin(6) who reported that oral harmine (10 mg/kg, 1 hour pretreatment) failed to increase brain serotonin levels, whereas this dose of harmine administered intraperitoneally effected a significant increase in brain serotonin. The ED_{50} for oral harmine as a tryptamine potentiator was found to be 4 mg/kg. Attention is directed to the fact that time of peak effect for the ED_{50} was 15 minutes after drug administration. Significant potentiation of tryptamine was no longer detectable 1 hour after drug administration. It is conceivable, therefore, that the aforementioned investigators might also have detected a significant elevation of brain serotonin levels by the oral route had they employed a shorter pretreatment time.

Although relative MAO inhibitory potencies of drugs measured by the tryptamine potentiation test generally parallel their relative potencies by biochemical procedures, some differences were observed. For example, Horita(10) reported that *dl*-PIH was approximately 20 to 40 times as potent as iproniazid, whereas *dl*-PIH was only 9 times as potent as iproniazid as a tryptamine potentiator. Horita and Parker(15) indicated that isonicotinyl-PIH was approximately twice as potent as iproniazid, whereas in the present studies it was found to be only one-half as potent. Finally, Chessin *et al.*(13) found phenelzine to be approximately 8 times as potent as iproniazid, whereas the data presented here indicate that these compounds do not differ significantly in tryptamine potentiation potency. Differences in routes of administration, pretreatment times, species, etc., may largely account for these variations. However, one other factor should be considered. In the usual biochemical procedure employed for measurement of *in vivo* MAO inhibition, homogenization of tissues removed from animals pretreated with an MAO inhibitor may result in a greater interaction between inhibitor and enzyme system than may occur in the intact animal. Consequently, compounds which because of their physical or chemical properties would ordinarily come into relatively poor contact with MAO may appear to

TABLE II. Compounds Which Failed to Effect Significant Potentiation of Convulsant Effects of Tryptamine in Rats.

Compound	Time of test	Dose, mg/kg (oral)	% potentiation
Diphenhydramine	2 hr	150	43
Tripelennamine	½-3 "	100	0
Isoniazid	3 "	100	0
Dihydroergotamine	1-2 "	30(s.c.)	0
Mescaline	½-3 "	200	0
SKF 525-A	1-5 "	200	0
SKF 5-A	15 min.	15	0
Procaine	½-3 hr	100	0
Procaine amide	½-3 "	100	0

be more effective after homogenization, when such contact would be enhanced.

Costa(8) reported SKF *cis* 385 to be less active than the *trans* isomer as an *in vivo* inhibitor of MAO, although no quantitative data were presented. This finding agrees with the tryptamine potentiating potency of this compound; SKF *cis* 385 being approximately one-half as potent as the corresponding *trans* isomer. Biel and co-workers(9) have indicated that the dextro-isomer of PIH is approximately 50% more potent than the racemate *dl*-PIH as an *in vivo* inhibitor of MAO. Although *d*-PIH was found to be twice as potent as the racemate as a tryptamine potentiator, comparison of the respective ED_{50}'s revealed that this difference in potency was not statistically significant ($P > .05$).

Although Tickner(16) reported that diphenhydramine and tripelennamine were effective *in vitro* inhibitors of MAO, the *in vivo* data presented here do not support this conclusion. Diphenhydramine, for example, potentiated tryptamine only at barely subtoxic doses, whereas tripelennamine failed completely to potentiate tryptamine. It is concluded that these compounds as well as the other compounds listed in Table II are not selective *in vivo* inhibitors of MAO.

Summary. The *in vivo* monoamine oxidase (MAO) inhibitory potency of a number of drugs was tested by measuring their activity as potentiators of the convulsant effects of tryptamine. Effective MAO inhibitors, arranged in descending order of potency, included harmine (s.c.) tranylcypromine, SKF *cis* 385, *d*-PIH, *dl*-PIH, harmine (oral), RO-4-1018, nialamide, phenelzine, iproniazid, and isonicotinyl-PIH. Compounds ineffective as MAO inhibitors included diphenhydramine, tripelennamine, isoniazid, dihydroergotamine, mescaline, SKF 525-A, SKF 5-A, procaine, and procaine amide.

1. Tedeschi, D. H., Tedeschi, R. E., Fellows, E. J., *Fed. Proc.*, 1959, v18, 450.
2. ———, *J. Pharmacol. and Exp. Therap.*, 1959, v126, 223.
3. Litchfield, J. T., Jr., Wilcoxon, F., *ibid.*, 1949, v96, 99.
4. Udenfriend, S., Witkop, B., Redfield, B. G., Weissbach, H., *Biochem. Pharmacol.*, 1958, v1, 160.
5. Pletscher, A., Besendorf, H., Bächtold, H. P., Gey, K. F., *Helv. Physiol. Pharmacol. Acta.*, 1959, v17, 202.
6. Tabachnick, I. I. A., Rubin, A. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 435.
7. Tedeschi, R. E., Tedeschi, D. H., Cook, L., Mattis, P. A., Fellows, E. J., *Fed. Proc.*, 1959, v18, 451.
8. Costa, E., *The Pharmacologist*, 1959, v1, 82.
9. Biel, J. H., Conway, A. C., DiPierro, F., Drukker, A. E., Nuhfer, P. A., *J. Am. Chem. Soc.*, 1959, v81, 4995.
10. Horita, A., *J. Pharmacol. and Exp. Therap.*, 1958, v122, 176.
11. Zeller, P., Pletscher, A., Gey, K. F., Gutmann, H., Hegedüs, B., Straub, O., *Ann. N. Y. Acad. Sci.*, 1959, v80, 555.
12. Rowe, R. P., Bloom, B. M., P'an, S. Y., Finger, K., *Fed. Proc.*, 1959, v18, 441.
13. Chessin, M., Dubnick, B., Leeson, G., Scott, C. C., *Ann. N. Y. Acad. Sci.*, 1959, v80, 597.
14. Zeller, E. A., Barsky, J., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 459.
15. Horita, A., Parker, R. G., *ibid.*, 1958, v99, 617.
16. Tickner, A., *Brit. J. Pharmacol.*, 1951, v6, 606.

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Action of Digoxin and Insulin on Transport of Glucose through Myocardial Cell Membrane. (25634)

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A decrease in myocardial glucose utilization has been found to be associated with failure in the dog heart-lung preparation(1). Administration of glucose or insulin results in an increase in myocardial glucose utilization and a restoration of the competency of the preparation(2). Administration of lanatoside C to the failing heart-lung preparation has also