Biochemical Pharmacology, 1958, Vol. 1, pp. 160-165.

STUDIES WITH REVERSIBLE INHIBITORS OF MONOAMINE OXIDASE: HARMALINE AND RELATED COMPOUNDS

SIDNEY UDENFRIEND, BERNARD WITKOP, BETTY G. REDFIELD and HERBERT WEISSBACH

Laboratory of Clinical Biochemistry, National Heart Institute, and
Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health, Public Health Service,
U.S. Department of Health, Education, and Welfare, Bethesda, Maryland.

(*Received* 21 *July*, 1958)

Abstract—Harmaline and related compounds have been shown to be potent inhibitors of monoamine oxidase effecting 50 per cent inhibition of serotonin metabolism at 10^{-6} M. Inhibition is reversible both *in vitro* and *in vivo*. Procedures have been presented for evaluating the effectiveness of monoamine oxidase inhibitors both *in vitro* and *in vivo*.

INHIBITORS of the enzyme monoamine oxidase (MAO) are no longer mere research tools but are finding application in therapeutics, particularly in the psychological and cardiovascular disorders. The compound which has been most widely used in this respect is isopropyl-isonicotinyl hydrazine (iproniazid).[1] As a result of the clinical findings with this compound there has developed a great interest in MAO inhibitors, and many new compounds of this type are being introduced.

The majority of these new MAO inhibitors are hydrazine derivatives, in analogy to iproniazid. There is no doubt that many more potent agents will be found with the hydrazine element acting as the "prosthetic group". In fact, one such compound, β-phenylisopropylhydrazine (PIH), has many times the activity of iproniazid.[2] However, the inhibition produced by the hydrazines is not a competitive and reversible one, and it has been shown that the effects of iproniazid last for many days following the disappearance of the drug from the body.[3] No doubt such long lasting, irreversible effects are in many cases desirable. However, from the experimental as well as the therapeutic standpoint it would be equally desirable to obtain reversible MAO inhibitors with short action *in vivo*. Potent reversible inhibitors have been described;[4] of these, the harmala alkaloids are the most active. This paper describes the actions of one of the harmala alkaloids, harmaline, both *in vitro* and *in vivo*, and presents the procedures which may prove generally useful for the laboratory evaluation of MAO inhibitors.

METHODS

Monoamine oxidase assays. These were carried out by measuring the disappearance of serotonin, as previously reported.[5] Serotonin was assayed spectrophotofluorometrically by the procedure of Bogdanski *et al.*[6] Harmaline is also a fluorescent base

and is extracted together with serotonin. Fortunately, its fluorescent characteristics in 3 *N* HCl are quite different from those of serotonin (activation maximum: serotonin 295 mμ, harmaline 380 mμ; fluorescence maximum: serotonin 550 mμ, harmaline 480 mμ). When 5–10 mg of harmaline were injected into rats the amounts of harmaline in the tissues were not sufficient to interfere with the serotonin assay. When larger amounts of harmaline were used, it was necessary to extract the alkalinized tissues with 15 ml of $CHCl_3$ before carrying out the usual *n*-butanol extraction for serotonin.

Harmaline assay. One ml of tissue homogenate in a glass-stoppered centrifuge tube was made alkaline by addition of 0·1 ml of 20 per cent Na_2CO_3 and 2 ml of 0·5 *M* borate buffer, pH 10. Fifteen ml of $CHCl_3$ were added and the tube was shaken and then centrifuged. Ten ml of the $CHCl_3$ fraction were transferred to another tube containing 2 ml of 0·1 *N* HCl. After agitation and centrifugation the aqueous layer was transferred to a cuvette and its fluorescence measured in the spectrophotofluorometer (activation 380 mμ, fluorescence 480 mμ) and compared to standards carried through the entire extraction procedure. Recoveries of harmaline added to tissues were quantitative. Further details of procedures for assay of harmala alkaloids will be published elsewhere.[7]

RESULTS

Inhibition of MAO in tissue homogenates

The effects of harmaline and related harmala alkaloids (Fig. 1) on MAO of rat liver are shown in Table 1. A comparison is made with iproniazid and PIH. It is apparent

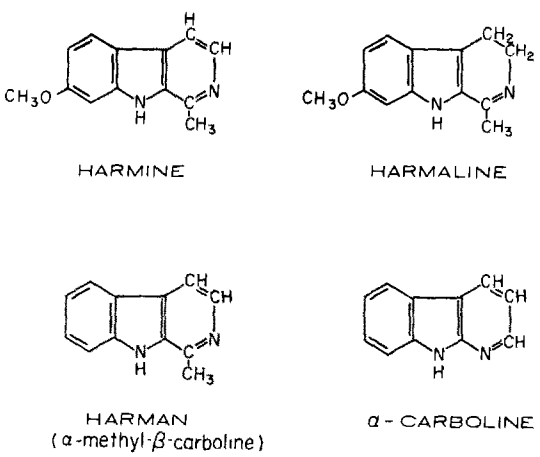

FIG. 1. Harmaline and related compounds.

that the harmala compounds are much more potent than either of the hydrazine compounds. However, direct comparison is rendered difficult in view of the different nature of the inhibitions. Iproniazid must be preincubated with enzyme under oxygen in order to be effective,[8] whereas harmaline, being a competitive inhibitor, needs no such preincubation. Following 20 minutes of preincubation with mitochondria the

TABLE 1. INHIBITION OF MAO IN HOMOGENATES OF RAT LIVER

Compound	Inhibition at 10^{-3} M per cent	Conc. producing 40–60 per cent inhibition
Harmaline	100	10^{-6}
Harmine	100	10^{-6}
1, 2, 3, 4-Tetrahydroharmine	97	10^{-5}
Harman	98	5×10^{-6}
D, L-Tetrahydroharman-3-carboxylic acid	0	—
1-3, 4-Dihydroharman-3-carboxylic acid	21	—
α-Carboline	47	—
β-Phenylisopropylhydrazine	100	5×10^{-6}
Iproniazid	90	3×10^{-4}

Procedure: The incubation mixture contained 1·5 ml. of rat liver homogenate (equivalent to 500 mg. of tissue), 0·5 ml. of 0·05 M phosphate buffer (pH 7·4), 1 mg. of serotonin, inhibitor and water to yield a total volume of 3·5 ml. Incubation was carried out in air at 37° C. for 40 minutes.

inhibition produced by iproniazid could not be reversed by washing the particles with saline. However, the inhibition produced by harmaline was readily reversed by this procedure (Table 2).

TABLE 2. REVERSIBILITY OF HARMALINE INHIBITION

Compound	Per cent inhibition	
	Before washing	After washing
Harmaline 10^{-5} M	92	38
Iproniazid 10^{-3} M	84	79

Procedure: Rat liver mitochondria (equivalent to 10 gm of liver) were incubated with the inhibitor at 37° C for 30 min at pH 7·4. The mitochondria were then washed four times with 5 ml of isotonic KCl. Control omitting inhibitor were incubated and washed in the same manner. Following this the washed mitochondria were made up to the original volume. Mitochondria equivalent to 1·5 gm of liver were then incubated with serotonin as described in Table 1.

Effects of harmaline in vivo

The following experiment was designed to investigate the ability of harmaline to inhibit MAO *in vivo* and to determine the duration of its effects. Harmaline was administered to rats intraperitoneally. At stated intervals several animals were killed. The livers and brains were homogenized and assayed for both MAO activity and harmaline content.

As shown in Fig. 2, MAO inhibition was strong for 2–3 hr and fell to negligible levels within 6 hr. As would be expected for a reversible inhibitor the harmaline levels in the tissues parallelled the degree of inhibition. This is quite different from the

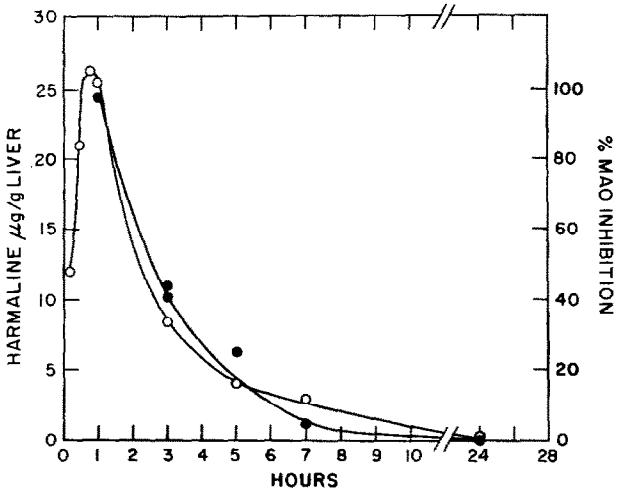

FIG. 2. Relationship between tissue levels of Harmaline and MAO inhibition. Harmaline was administered intraperitoneally (5 mg/kg) into rats and the animals were killed at stated intervals. The livers were removed and homogenized in water (3° C): 1 g of liver plus 2 ml of water. Aliquots were taken for assay of MAO activity as described in Table 1 and for harmaline assay.

○——○ Harmaline; ●——● MAO Inhibition

effects observed with iproniazid where the inhibition persists for many days following the disappearance of the compound from the body.[3]

Effect of harmaline on conversion of serotonin to 5-hydroxyindoleacetic acid (5HIAA) in vivo

One mg of serotonin was injected intraperitoneally into mice (18–20 g); these included controls and animals which had been given harmaline (10 mg/kg) intravenously 10 minutes before the experiment. At the end of one hour the mice were homogenized* and assayed for both serotonin and 5HIAA. It is apparent (Table 3) that

TABLE 3. EFFECT OF HARMALINE ON CONVERSION OF SEROTONIN TO 5HIAA IN THE INTACT MOUSE

Exp.	Interval following serotonin injection	Serotonin metabolized	5HIAA formed
	hr	mg/animal	mg
Control animals	1	1·68	0·23
	2	1·76	0·23
Harmaline animals	1	1·20	0·02
	2	1·60	0·03

Mice were given 15 mg of harmaline per kg intraperitoneally. After one hour these animals and untreated controls were given 2 mg of serotonin intraperitoneally. The animals were killed at stated intervals following the serotonin injection and homogenized in 100 ml of 0·1 N HCl and assayed for serotonin and 5HIAA.

* Urine and faeces produced during the experimental period were included in the homogenization procedure.

although equivalent amounts of serotonin disappeared in both cases the conversion to 5HIAA was markedly inhibited in those animals pretreated with harmaline.

Effect of harmaline on endogenous levels of amines

If a MAO inhibitor is effective it can increase levels of endogenous amines in tissues. The level of brain serotonin is particularly sensitive to the effects of MAO inhibitors, as was previously shown with iproniazid.[9] When harmaline was administered to rats in doses of 5–15 mg/kg the brain serotonin level was found to increase (Fig. 3). The

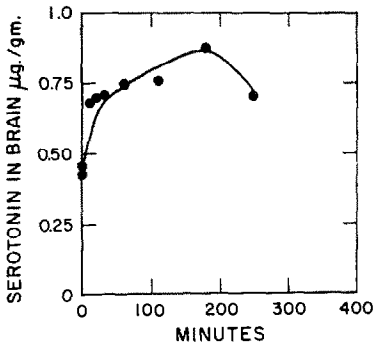

FIG. 3. Effect of Harmaline on Brain Serotonin Levels. Harmaline was administered in doses ranging from 5–15 mg/kg. Animals were killed at stated intervals and the brains were assayed for serotonin. The 0-time control value represents the average obtained on 27 animals. From 10–60 min the values represent the averages obtained with 9 animals. Subsequent figures represent the averages obtained on 6 animals.

magnitude of the increase indicates that harmaline can effectively inhibit the metabolism of serotonin. The rapidity of the increase may be taken as an index of the rapid turnover of serotonin in the brain.[10]

DISCUSSION

If one compares the inhibitors of MAO with those of cholinesterase then harmaline can be compared in its reversible mode of action to physostigmine, and iproniazid to the irreversible inhibitor, di-isopropyl fluorophosphate (DFP). In the laboratory both types of MAO inhibitors will prove useful. However, it should be kept in mind that comparisons of reversible and irreversible agents *in vivo* are not a simple matter. Each agent has its advantages and disadvantages. From a clinical standpoint, long-lasting effects may be desirable. However, we know little about the function of MAO and of the possible clinical consequences of its inactivation for long periods of time. Furthermore, hydrazines will most likely react irreversibly with other biological sites. Experience with cholinesterase inhibitors has shown that the reversible agents such as prostigmine are clinically far safer to use and easier to control.

It is of interest that harmaline, harmine and other harmala alkaloids were used therapeutically as early as 1920, for various disorders for which iproniazid is now suggested.[11] This was before MAO was even known. That harmaline does effectively inhibit MAO in man, in doses which have been used therapeutically, has been shown by Sjoerdsma, Gillespie and Udenfriend.[12] Many of the effects reported here for harmaline have already been observed with harmine, harman and tetrahydronorharman. These and other analogues may prove to be as interesting as harmaline.

The limited number of representatives of carboline compounds, tested hitherto for MAO inhibition, does not offer a simple rationale for the construction of active inhibitors. However, a few guiding principles become discernible at this time: (i) fully aromatic β-carbolines are very active, while α-carboline is relatively inactive; (ii) di- and tetrahydro-β-carbolines are active (the fact that they contain the elements of a tryptamine substituted in the α-indole position and at the nitrogen in the side chain may contribute to their activity as competitive inhibitors); (iii) substitution in the benzene ring or at C(2) in the pyridine ring does not significantly alter the degree of inhibition; and (iv) the α-amino acid "precursors" of tetrahydroharman and of harmaline are inactive.

In these circumstances the re-investigation of the therapeutic possibilities of this class of compounds, utilizing dosages which effectively inhibit MAO, is acquiring new interest. Whether or not the harmala alkaloids turn out to be clinically useful these studies indicate that it is possible to obtain potent reversible MAO inhibitors which function as such *in vivo* both in experimental animals and in man.

The methods used to detect and quantitate the effectiveness of MAO inhibitors presented here are quite simple. They should facilitate the search for new and more active MAO inhibitors. When used in conjunction with the newly devised serotonin metabolism test,[12] inhibitors may be found which will also be therapeutically useful.

Acknowledgments—The activities of several MAO inhibitors were determined by Dr. Masayori Ozaki who is continuing further studies along these lines. Harmaline and harmine were kindly supplied through the courtesy of Dr. F. A. Hochstein, Chas. Pfizer and Co. D:L-Tetrahydroharmon-3-carboxylic acid and 1-3-4-di hydroharman-3-carboxylic acid were put at our disposal by Professor R. Tschesche, Hamburg. Iproniazid was supplied by Hoffman-La Roche, New Jersey, and β-phenylisopropylhydrazine by Lakeside Laboratories, Wisconsin.

REFERENCES

1. Symposium on Iproniazid, *J. Clin. Exptl. Psychopathol.* Supplement, **19**, (1958).
2. A. HORITA, *Fed. Proc.* **17**, 379 (1958).
3. S. HESS, H. WEISSBACH, B. G. REDFIELD and S. UDENFRIEND, *J. Pharmacol.* In Press.
4. K. FRETER, H. WEISSBACH, B. G. REDFIELD, S. UDENFRIEND and B. WITKOP, *J. Amer. Chem. Soc.* **80**, 983 (1958).
5. A. SJOERDSMA, T. E. SMITH, T. D. STEVENSON and S. UDENFRIEND, *Proc. Soc. Exp. Biol., N. Y.* **89**, 36 (1955).
6. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmacol.* **117**, 82 (1956).
7. S. HESS, *et al.*, to be published.
8. A. N. DAVISON, *Biochem. J.* **67**, 316 (1957).
9. S. UDENFRIEND, H. WEISSBACH and D. F. BOGDANSKI, *J. Pharmacol.* **120**, 255 (1957).
10. S. UDENFRIEND and H. WEISSBACH, *Proc. Soc. Exp. Biol., N. Y.* **97**, 748 (1958).
11. J. A. GUNN, *Arch. Int. Pharmacodyn.* **50**, 379 (1935).
12. A. SJOERDSMA, L. A. GILLESPIE and S. UDENFRIEND, *Lancet.* **2**, 159 (1958).