Biochemical Pharmacology, 1964, Vol. 13, pp. 371–378. Pergamon Press Ltd., Printed in Great Britain.

AN ANALYSIS OF THE INTERACTION OF REVERSIBLE AND IRREVERSIBLE MONOAMINE OXIDASE INHIBITORS*

A. HORITA and C. CHINN

Department of Pharmacology, School of Medicine,
University of Washington, Seattle, Wash., U.S.A.

(*Received* 15 *July* 1963; *accepted* 1 *October* 1963)

Abstract—The interaction of the reversible and irreversible inhibitors of monoamine oxidase (MAO) in the intact rat has been investigated. The results indicate that the extent of antagonism of the irreversible hydrazine inhibitors by harmine or harmaline depends upon two factors: (1) the duration of action of the reversible inhibitor, and (2) the duration of the presence of the irreversible inhibitor in the supernatant fraction (cytoplasmic or interstitial fluid) of the tissue. The duration of harmine was about one-half as long as that of harmaline. Harmine was also less effective as an antagonist of iproniazid. Pheniprazine (PIH) activity disappeared from the supernatant fraction of liver and brain homogenates prepared from rats within 60 to 90 min, whereas iproniazid activity was present for 12–18 hr after administration. It appears that a complete antagonism of PIH by harmine or harmaline results because the reversible inhibitors protect the enzyme from PIH for the duration of its presence. The action of iproniazid is only partly antagonized because it is present in the supernatant for longer periods of time than the reversible inhibitors.

THE accumulation of 5-hydroxytryptamine and catecholamines in brain after administration of the long-acting inhibitors of monoamine oxidase (MAO) has been employed frequently as an index of inhibition of brain MAO in the intact animal. After pretreatment of rats with a short-acting inhibitor of MAO, such as harmaline or harmine, this property of the hydrazine-type inhibitors of MAO may be prevented.[1] Direct assays of brain and liver MAO after treatment with harmine and followed with β-phenylisopropylhydrazine (JB-516, PIH) also resulted in a complete antagonism of the usual inhibitory actions of PIH.[2] However, pretreatment of the animal with harmine did not prevent the inhibition of the enzyme as produced by the nonhydrazine compound, 2-phenylcyclopropylamine (SKF-385, tranylcypromine). The authors concluded that harmine prevented the inhibitory action of PIH by occupying the active site of the enzyme and preventing its access to PIH. The inability of harmine to block the action of SKF-385, however, was considered to be the result of the displacement of harmine by SKF-385 and itself occupying the active site of MAO.

In similar studies Ehringer *et al.*[3] attempted to correlate the lack of increase of brain amines with the antagonism of the MAO-inhibiting action of the hydrazine compounds when rats were pretreated with either methylene blue or harmine, followed by iproniazid or nialamide. Although MAO of brain was completely inhibited under

* This investigation was supported in part by Grant MH-02435-05 from the National Institute of Mental Health, Public Health Service.

these conditions, they found that levels of 5-hydroxytryptamine and norepinephrine did not rise. These results indicated the absence of a causal relationship between inhibition of MAO and the rise of amine levels in the brain. In contrast, Spector *et al.*[4] found harmaline effective in blocking the prolonged action of iproniazid on MAO and on its ability to reverse the effects of reserpine. Our earlier work also demonstrated complete antagonism of the anti-MAO action of PIH when animals were pretreated with harmine. Since both PIH and iproniazid are hydrazines and presumably act by an identical mechanism, the differences in results were not clearly understood.

The present study was therefore undertaken to clarify this discrepancy and also to investigate further the nature of the interaction of the reversible and irreversible MAO inhibitors.

METHODS

Male Sprague-Dawley rats weighing 100–200 g were used throughout these experiments. All doses of the drug are expressed as the base and were injected by the intraperitoneal route. Harmine or harmaline was administered 30 min prior to the long-acting inhibitors.† After the designated times the animals were sacrificed, and their brains were assayed for MAO activity according to the method of Udenfriend *et al.*[5] Brain serotonin levels were also measured in some of these animals, by the spectrophotofluorimetric assay of Bogdanski *et al.*[6]

In part of the study the supernatant fraction of brain homogenates prepared from rats pretreated with PIH or iproniazid was assayed for anti-MAO activity. This procedure consisted of preparing a 33% homogenate, centrifuging it at 40,000 *g* for 30 min and assaying the supernatant fractions for anti-MAO activity on a rat liver mitochondrial preparation. Further details of the procedure are found in an earlier report.[7] As indicated there, the "supernatant fraction" contains the soluble material and some microsomes. This fraction was found to have no apparent effect by itself on the inhibitors or on the mitochondrial MAO preparation.

RESULTS

Duration of MAO inhibition by reversible inhibitors

The intraperitoneal administration of equal doses of harmaline or harmine (30 mg/kg) resulted in a rapid and complete inhibition of brain MAO. The duration of action of the two compounds, however, was quite different in that the harmaline effect was approximately twice as long (Fig. 1). Thus, 4 hr after administration, the brain MAO of harmaline-pretreated animals exhibited about an 80 per cent inhibition, whereas the harmine animals displayed less than 20 per cent inhibition of brain MAO. Complete recovery from the harmaline effect required some 12–16 hr, whereas harmine effects disappeared within 8 hr. In an attempt to determine the nature of the rapid disappearance of action of these inhibitors some animals were pretreated with SKF-525A (β-diethylaminoethyl-diphenylpropylacetate) in a dose of 50 mg/kg, and this was followed 15 min later with the harman derivatives. In all instances the duration of MAO inhibition was greatly lengthened, indicating that the microsomal 'drug-detoxifying' enzyme system was responsible for the inactivation of the harmala alkaloids that inhibit MAO.

† Pheniprazine (Catron, PIH) and iproniazid (Marsilid) were kindly supplied by the Lakeside Laboratories, Inc., Milwaukee, Wis., and the Hoffman-La Roche Co., Nutley, N.J., respectively.

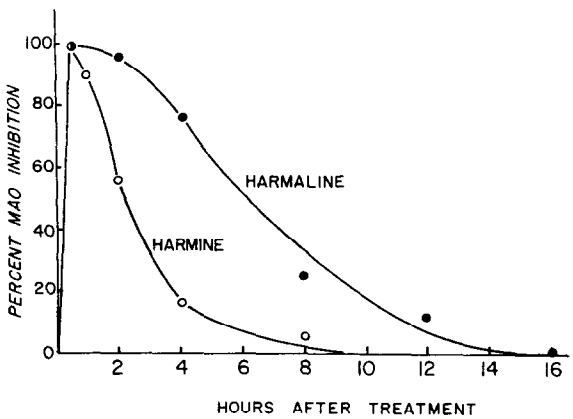

FIG. 1. A comparison of the duration of MAO inhibition in rat brain after administration of 30 mg harmaline or harmine/kg. Each point represents the mean of 3 to 5 determinations.

Duration of presence of the long-acting MAO inhibitor in brain supernatant

The inhibition of MAO by the hydrazine compounds is irreversible, and the duration of action could not be correlated with the duration of inhibitor concentration at any given time. However, by analysing the relative concentrations of anti-MAO activity present in the supernatant fraction of brains from rats pretreated with the inhibitor, it was possible to determine differences in the character of the disappearance of inhibitor from the tissue. A detailed account of such experiments has been published elsewhere, and the results of the present study are essentially the same (Table 1). In brief, the supernatant fractions of brains from rats pretreated with PIH in doses up to

TABLE 1. THE RATE OF DISAPPEARANCE OF MAO INHIBITOR ACTIVITY IN SUPERNATANT OF BRAIN HOMOGENATES FROM RATS PRETREATED WITH IPRONIAZID (100 mg/kg) AND PHENIPRAZINE (10 mg/kg)

Each value represents a single experiment.

Time	Inhibition of mitochondrial MAO by PIH, %	Iproniazid, %
5 min	92, 95, 92	
10 ,,	64, 82, 72	
30 ,,	8, 10, 18, 20	30, 35, 38
1 hr	4, 4, 11, 12	33, 55, 58
2 ,,		44, 50, 56
4 ,,		36, 52, 55
6 ,,		30, 32, 36, 42
12 ,,		14, 16, 20
18 ,,		10, 15, 18

10 mg/kg demonstrated a rapid rise in anti-MAO activity. This activity fell equally rapidly, and within 60 to 90 min essentially no inhibitory activity remained, although brain MAO was completely inhibited. The administration of iproniazid (100 mg/kg), however, resulted in a gradual increase in supernatant MAO inhibitor activity, the peak concentration being reached in 60–120 min. Thereafter, anti-MAO activity decreased slowly, but complete elimination of supernatant anti-MAO activity required at least 18 hr after iproniazid administration.

Interaction of reversible and irreversible inhibitors

When PIH was administered 30 min after harmine or harmaline and the time–effect relationship observed, it was evident that the normal PIH inhibition curve was absent. Both of the harmala alkaloids were effective in completely antagonizing the anti-MAO action of PIH, as was shown in our earlier work.[2]

However, iproniazid presented an entirely different picture (Fig. 2). Iproniazid alone (25 mg/kg) exerted complete inhibition of brain MAO within 4–6 hr and maintained this effect beyond 24 hr. In the harmine-pretreated rats iproniazid followed

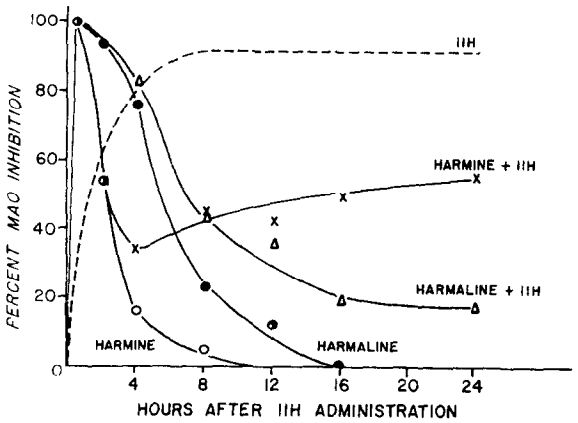

FIG. 2. Graph representing the pattern of inhibition of brain MAO in rats after treatment with 30 mg harmaline or harmine/kg or 25 mg iproniazid/kg, or their combinations as indicated in the figure. Each point represents the mean determination on 3 to 6 rat brains.

the curve of the reversible inhibitor for the first 2 hr. Thereafter the control harmine effect falls rapidly, but the harmine–iproniazid curve shows a reverse trend and inhibition begins to increase. At the end of 24 hr animals with the harmine–iproniazid injections demonstrated some 55 per cent inhibition of their brain MAO. As indicated in Fig. 1, harmaline inhibited MAO for a considerably longer duration. Iproniazid administered 30 min after harmaline produced results that resembled those seen with harmine–iproniazid, except that the time–effect curve deviated from that of harmaline at about 8 hr and continued to show some brain MAO inhibition throughout the 24-hr test period. The degree of MAO inhibition throughout the time course of 8–24 hr after the administration of iproniazid was considerably less than that observed in the harmine–iproniazid group, demonstrating that harmaline was more effective in protecting MAO from the irreversible effect of iproniazid.

Dose–effect relationship and levels of brain serotonin

The MAO activity and serotonin levels in brains of rats treated with varying doses of iproniazid and PIH in the presence and absence of harmaline were also determined (Table 2). Harmaline, 10–30 mg/kg, was administered 30 min prior to iproniazid or PIH, and 16 hr later the brains were assayed. In the control rats a dose of 25–50 mg/kg iproniazid was necessary to produce complete inhibition of brain MAO. Brain serotonin levels, however, progressively increased with higher doses of iproniazid,

but at doses above 300 mg/kg they appeared to develop a plateau in the concentration curve. In the harmaline-pretreated animals the dose–MAO inhibition curve was markedly changed, and within the doses used in these experiments complete inhibition of brain MAO was not possible. Inhibition was minimal at 10–20 mg/kg, and at 200 mg/kg some 80 per cent inhibition was evident. The increase in brain serotonin levels was also attenuated in the animals pretreated with harmaline. With 100 mg/kg iproniazid only a 40 per cent increase in serotonin levels was observed as compared

TABLE 2. THE INFLUENCE OF HARMALINE (10 mg/kg) ON THE INHIBITION OF RAT BRAIN MAO AND 5-HYDROXYTRYPTAMINE (5HT) LEVELS EXERTED BY IPRONIAZID

Values are expressed as per cent MAO inhibition or 5HT increase and represent the means of 4–6 experiments. The numbers in parentheses indicate the range of the values.

Dose of iproniazid, mg/kg	Iproniazid		Harmaline + iproniazid	
	MAO inhibition, %	5HT increase, %	MAO inhibition, %	5HT increase, %
25	95 (92–97)	80 (70–89)	27 (25–29)	0 (0)
75	100 (100)	121 (110–133)	51 (41–62)	28 (15–45)
100	100 (93–100)	113 (77–136)	60 (51–70)	44 (36–67)
200	100 (97–100)	230 (210–250)	79 (69–83)	46 (36–67)
300	100 (97–100)	285 (264–310)	84 (77–95)	89 (60–140)

to 120 per cent in control animals. Even with doses as high as 300 mg/kg, brain levels of serotonin did not increase by much more than 100 per cent above control levels. In other experiments the harmaline dosage was increased to 20 mg/kg, and in these instances there resulted a further shift of the dose–response curve to the right, indicating greater antagonism of the iproniazid-induced inhibition of MAO.

TABLE 3. THE INFLUENCE OF HARMALINE (10 mg/kg) ON THE INHIBITION OF RAT BRAIN MAO AND 5-HYDROXYTRYPTAMINE (5HT) LEVELS EXERTED BY PHENIPRAZINE (PIH)

Values are expressed as per cent MAO inhibition or 5HT increase and represent the means of 4–6 experiments. The numbers in parentheses indicate the range of the values.

Dose of pheniprazine, mg/kg	Pheniprazine		Harmaline + Pheniprazine	
	MAO inhibition, %	5HT increase, %	MAO inhibition, %	5HT increase, %
1	89	42 (33–50)	0	0
2	90 (84–93)	125 (125)	7 (2–11)	0
4	100	114 (110–118)	12 (4–16)	8 (8)
8	100	135 (100–182)	20 (15–27)	8 (0–33)
12	100	132 (128–140)	29 (17–47)	16 (0–30)
16	100	171 (130–200)	36 (33–41)	23 (7–33)

The antagonism of PIH by harmaline was even more pronounced than that exerted against iproniazid. In the control animals complete MAO inhibition in brain resulted after doses of 1 mg/kg PIH. Some increase in brain serotonin was evident at this dose,

but maximal increases were obtained with doses above 8 mg/kg. In the harmaline-pretreated rats it was essentially impossible to achieve any degree of MAO inhibition or an increase in brain levels of serotonin (Table 3). Even with doses as high as 16 mg/kg, PIH was effective in inhibiting brain MAO only some 36 per cent and with less than 20 per cent increase in serotonin levels. Higher doses of PIH given to harmaline-pretreated animals were extremely toxic, and a large proportion of animals succumbed when these conditions were employed.

DISCUSSION

The antagonism of the hydrazine-type inhibitors of MAO by harmine or harmaline is generally explained on the basis of competition for the active site of the enzyme. The reversible inhibitors presumably combine with the MAO in such a manner as to prevent its access to the irreversible inhibitor. However, two important factors have not been considered in such studies: (1) the duration of action of the reversible inhibitors, and (2) the duration of presence of the irreversible inhibitors in the tissues assayed.

Although harmine and harmaline are very similar in chemical structure and in biological activity, the present studies indicate that their duration of action in inhibiting MAO in the intact animal is quite different. Harmaline possesses approximately twice the duration of action of harmine. This difference is probably a matter of different rates of degradation by the microsomal enzyme system, since compound SKF-525A prolongs markedly the duration of action of both agents.

As was shown in an earlier study and repeated here, the hydrazine-type inhibitors differ in their ability to remain in tissues in an active form. For instance, much higher levels of MAO inhibitor activity were found in the supernatant fraction of brains of rats pretreated with PIH, but this was only transient, for within an hour most of this activity had disappeared. However, iproniazid, while not achieving excessively high inhibitor activity, persisted for over 12 hr in measurable quantities in the supernatant fraction of brain. The earlier work indicated a rapid inactivation or binding of PIH with tissue components, whereas iproniazid persisted unaffected in the cytoplasm or extracellular fluid of the tissue.

These findings are of importance in order to explain the discrepancy of results obtained by Horita and McGrath[2] and by Ehringer *et al.*[3] Our original work on the interaction of reversible and irreversible inhibitors involved harmine and PIH. It is possible to explain the antagonism of the irreversible agent on the basis of its being present in the tissue for a shorter period of time than the reversible inhibitor. Thus, both harmine and harmaline remain in the tissues for longer periods than does PIH, as is evident from the present studies. Increasing the dose of PIH will lengthen its duration to some extent, but even with 10 mg/kg, the MAO inhibiting activity of brain supernatant disappeared within 2 hr after administration. Harmine at this time still exerted some 60 per cent inhibitory activity whereas harmaline exhibited 100 per cent inhibition. It is therefore probable that PIH is inactivated, either through tissue binding or degradation, and a sufficient concentration is not present when the reversible inhibitor concentration decreases.

On the other hand, iproniazid remained in the brain for a prolonged period of time. Anti-MAO activity could be found in the supernatant fraction hours after the disappearance of the reversible inhibitors. The interaction of reversible and irreversible

inhibitors is therefore dependent in part on the duration of action of both types of inhibitors. This was shown by the fact that after harmine iproniazid exerted greater anti-MAO activity than after harmaline, the longer-acting reversible inhibitor. The results illustrated in Fig. 2 suggest that with the disappearance of the reversible inhibitor the residual quantity of iproniazid can inhibit the MAO. The extent of the inhibition is dependent upon the amount of irreversible agent present, which is in turn influenced by the duration of the reversible inhibition. Since iproniazid persists in an active form in the tissue for long periods of time, it is possible to overcome the antagonism as produced by harmine and harmaline. By increasing its dose sufficiently iproniazid may produce almost complete inhibition of brain MAO in the intact rat, even in the presence of harmaline, which was not possible with PIH. Brain levels of 5HT are also raised, but in all instances this action is attenuated as compared to control animals. The dose–response curve of iproniazid in the presence of harmaline indicates a shift of the curve to the right, so that in order to achieve the same extent of activity as with control animals, it is necessary to increase the dose of the iproniazid.

Although it is not reported in the results, it should be mentioned that the time interval between the injections of the reversible and irreversible inhibitors will also influence the extent of antagonism produced. The greater the interval, the less the antagonism exerted by the reversible inhibitor. It is reasonable to expect this, for with an increase in the interval between the reversible and irreversible inhibitors, there will be a lower concentration of the former to oppose the latter agent.

Thus, the results described in this investigation clarify the conflicting reports between Ehringer *et al.*[3] and this laboratory. The former authors employed harmine as the reversible agent and iproniazid as the irreversible inhibitor; 1 hr after harmine injection, iproniazid in a dose of 100 mg/kg was administered. This combination will in fact permit considerable inhibition of brain MAO to take place because of the prolonged presence of iproniazid in the tissue. In our studies PIH was used as the irreversible inhibitor and, as described in the earlier study, this compound persists in the supernatant fluid of brain tissue for a very short period. The reversible inhibitors are therefore present for longer periods than is PIH and thus protects the enzyme from the latter agent.

Our original report also demonstrated the inability of harmine to antagonize the MAO-inhibiting property of the nonhydrazine compound, tranylcypromine (SKF-385). Recent studies indicate that this inhibitor resembles iproniazid in that it persists in its active form in the tissues for considerable periods. It appears, therefore, that rather than replacing harmine from the enzyme, as suggested, tranylcypromine persists in tissues in sufficient concentration to exert its effect after the reversible inhibitor has disappeared. Also, in accordance with the explanations proposed, harmaline, possessing a longer duration of action than harmine, was found to be more effective in antagonizing tranylcyprominc.

In conclusion, the analysis of the antagonism of the irreversible inhibitors of MAO by harmine or harmaline has revealed that several factors are involved in this interaction. These include (1) the duration of action of the reversible inhibitor, and (2) the duration of presence of active irreversible inhibitor in the tissues. If the latter factor persists beyond the former, complete antagonism of the irreversible inhibitor is not possible. In contrast, if the reversible agent possesses a longer duration than the presence of the irreversible inhibitor, then complete antagonism may be produced.

REFERENCES

1. A. PLETSCHER and H. BESENDORFF, *Experientia (Basel)*, **15**, 25 (1959).
2. A. HORITA and W. R. MCGRATH, *Biochem. Pharmacol.* **3**, 206 (1960).
3. H. EHRINGER, O. HORNYKIEWIEZ and K. LECHNER, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **241**, 568 (1961).
4. S. SPECTOR, R. KUNTZMAN, P. A. SHORE and B. B. BRODIE, *J. Pharmacol. exp. Ther.* **130**, 256 (1960).
5. S. UDENFRIEND, H. WEISSBACH and B. B. BRODIE, *Meth. biochem. Anal.* **6**, 95 (1958).
6. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmacol. exp. Ther.* **117**, 82 (1956).
7. A. HORITA, *J. Pharmacol.* **142**, 141, (1963).