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THE INTERACTION BETWEEN REVERSIBLE AND IRREVERSIBLE MONOAMINE OXIDASE INHIBITORS*

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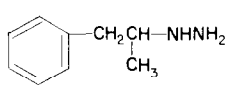
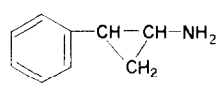
Abstract—The influence of the alkaloid harmine on the inhibition of monoamine oxidase (MAO) by β-phenyl*iso*propylhydrazine (PIH) and 2-phenyl*cyclo*propylamine (SKF-385) has been investigated. Rats pretreated with harmine were effectively protected from the long acting inhibitory effects of PIH, but not readily from those of SKF-385. The duration curves of the inhibition of MAO by PIH and SKF-385 in normal and harmine-pretreated animals were also compared. The animals treated with both harmine and PIH showed a short duration of action, typical of harmine alone, while those animals treated with both harmine and SKF-385 showed the usual SKF-385 duration curve. These findings suggest that PIH and harmine may act at the same site on the enzyme; however, the mechanisms of inhibition of MAO produced by PIH and SKF-385 may be quite different.

THE inhibition of the enzyme, monoamine oxidase (MAO), by a variety of hydrazine derivatives is in many ways similar to that produced by the organophosphorus compounds on cholinesterase (ChE). In both instances the inhibition is irreversible in nature, and when administered to intact animals both types of compounds lead to a prolonged inhibition of the respective enzymes.[1] The resulting pharmacological effects in these cases are presumably the result of an accumulation of the substrates in various areas of the body.



(JB – 516, PIH)



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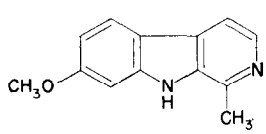

Harmine

An interesting phenomenon associated with the inhibition of ChE is its apparent protection from irreversible inhibition by the organophosphorus compounds through prior treatment with physostigmine, a reversible ChE inhibitor. This effect was first described by Koster[2] and the mechanism demonstrated by Koelle.[3]

The current interest in MAO has made available a variety of inhibitors of this enzyme. Several of these agents have been employed in the present study. The chemical

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structures of these compounds are shown below; they are: harmine, β-phenyl*iso*propylhydrazine (JB-516, PIH), and 2-phenyl*cyclo*propylamine (SKF-385). Harmine is a potent, but short-acting, reversible inhibitor of MAO. PIH has been described as an irreversible and long-acting inhibitor,[4] similar to a number of other hydrazine compounds; SKF-385, a new type of MAO inhibitor,[5] also possesses a relatively long duration of action. The present paper is concerned with the influence of the short acting inhibitor, harmine, on the activity of the longer acting agents. This study was made to determine whether reversible inhibitors of MAO could protect the enzyme from the effects of the longer acting inhibitors.

METHODS

Male Sprague-Dawley rats weighing 175–250 g were used throughout these experiments. All drugs, with the exception of harmine, were injected subcutaneously. Harmine, in doses of from 5 mg/kg to 60 mg/kg, was administered intraperitoneally 35–40 min prior to either PIH or SKF–385. The doses of 1 mg/kg (PIH) and 2 mg/kg (SKF–385) were selected because preliminary studies showed them to produce about the same degree of inhibition 16–18 hr after their administration.

At various intervals after injection of the long-acting inhibitor the animals were sacrificed. The livers and brains were quickly removed, ground with Teflon homogenizers, and diluted to concentrations of 20 per cent and 33 per cent, respectively. One ml of the homogenate was incubated with serotonin creatinine sulfate (4 μmoles) and phosphate buffer (pH 7·0) for periods of 30 and 60 min, respectively. The resulting mixture was then assayed for residual serotonin, using the nitroso-naphthol colorimetric method, as described by Udenfriend *et al.*[6]

The extent of MAO-inhibition was calculated by comparing the amount of serotonin metabolized by the treated and control homogenates. All data are expressed in per cent of MAO-inhibition.

RESULTS

Fig. 1 indicates the influence of prior treatment of rats with 20 mg of harmine per kg, on the inhibitory effects of PIH and SKF-385 on MAO. These results were obtained 16–18 hr after the injections of the inhibitors. This time interval was used since the harmine effect was completely absent, while the normal effects of PIH or SKF-385 were still markedly present. It is apparent that in those animals pretreated with harmine, PIH did not exert its effect. Control PIH animals showed an average of 86 per cent inhibition in brain and 65 per cent inhibition in liver, while tissues from harmine-pretreated animals showed essentially no inhibition. With SKF-385, there was no apparent difference between harmine-pretreated and the control animals. The MAO in both liver and brain, in harmine-pretreated animals, as well as in control animals, responded similarly to SKF-385; the inhibition observed was about 70 per cent inhibition in brain and 57 per cent in liver. Each of the bars in Fig. 1 is the mean value from five to ten animals.

When the harmine dose was lowered the degree of antagonism of the effect of PIH was decreased. As indicated in Table 1, doses of 10 and 5 mg of harmine per kg had progressively diminishing effects on the inhibition of MAO by PIH. However, even with the lowest dose used (5 mg/kg), harmine still exhibited a considerable antagonism of PIH. In contrast to PIH the antagonism of the effect of SKF-385 on MAO was

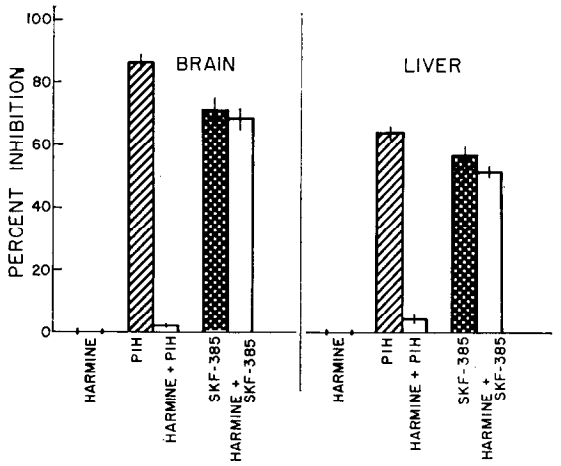

FIG. 1. The influence of harmine-pretreatment (20 mg/kg) on inhibition of brain and liver MAO by PIH and SKF-385. These values of MAO inhibition were obtained 16–18 hr after injection of the indicated agents. Each bar represents the mean of 5–10 experiments. Vertical lines indicate the S.E.M.

difficult to demonstrate even with toxic doses of harmine. Doses of 40 and 60 mg/kg were used, and at the highest dose the animals showed marked tremors and motor incoordination; four of the eight animals treated in this manner succumbed within 12 hr. Even with these high doses of harmine the effects of SKF-385 were not altered significantly in the liver; in the brain, however, some antagonism was shown, as can be seen in Table 1.

TABLE 1. THE EFFECT OF HARMINE ON THE INHIBITION OF MAO BY PIH AND SKF–385

Pretreatment with Harmine (mg/kg)	1 percent MAO inhibition by PHH[1] Brain	Liver
0	86 ± 3·0 (5)	64 ± 2·4 (6)
5	26 ± 5·9 (4)	27 ± 3·6 (3)
10	11 ± 2·0 (4)	13 ± 1·6 (4)
20	2 ± 0·1 (5)	5 ± 2·6 (6)
	by SKF–385	
0	71 ± 3·1 (9)	57 ± 3·0 (10)
20	68 ± 3·5 (5)	51 ± 2·4 (6)
40	53 ± 4·8 (6)	54 ± 4·4 (6)
60	48 ± 5·5 (4)	49 ± 3·9 (4)

The figures listed above are the mean values of the percent of MAO inhibition ± the S.E.M., the standard error of the means. Figures in parentheses indicate the number of experiments carried out at each dose level.

Figs. 2 and 3 demonstrate the duration of MAO-inhibition produced by these three compounds on the MAO of brain, and its modification by pretreatment with harmine. In both of these figures each point represents the average values from two animals. Harmine itself had a short duration of action. With the doses used in these experiments

(20 mg/kg), essentially complete inhibition of the MAO of brain was produced within 15 to 30 min after administration. Within 4 hr at least eighty per cent of the enzyme activity had returned, and after 12 hr recovery was complete. PIH, on the other hand, exerted a prolonged inhibition of MAO, as can be seen in Fig. 2. Even 48 hr after its

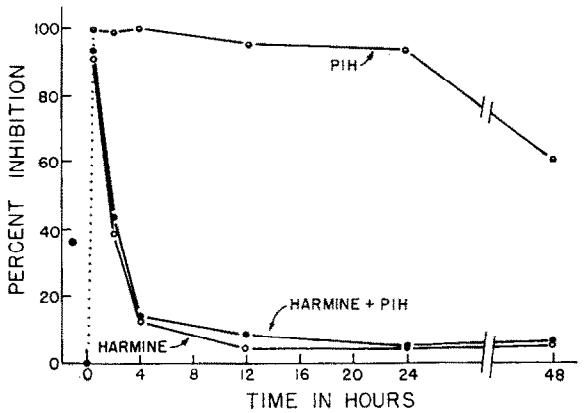

FIG. 2. The duration of action of harmine (20 mg/kg) and PIH (1 mg/kg) on brain MAO, and the effect of harmine-pretreatment on the duration of action of PIH. Drugs were administered at zero time. Each point represents the average values obtained from two animals.

administration there was still about a 60 per cent blockade of the brain enzyme. In the harmine pretreated animal, however, the long duration of action of PIH was completely absent. Instead of a prolonged action, PIH in these cases closely approximated the duration curve typical of harmine alone, which indicates that the latter agent had prevented the effects of PIH from being exerted.

In Fig. 3, SKF-385 is shown to exert a relatively long duration of action. Like PIH and harmine its onset is rapid, and there is a gradual decrease in its inhibitory effect over 24 and 48 hr. At the end of 48 hr there was still a 40–45 per cent inhibition of brain MAO. In harmine-pretreated animals the course of the inhibition by SKF-385

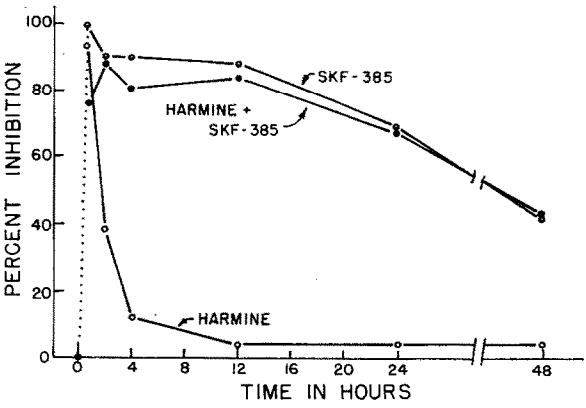

FIG. 3. The duration of action of harmine (20 mg/kg) and SKF-385 (2 mg/kg) on brain MAO, and the effect of harmine on the duration of action of SKF-385. Drugs were administered at zero-time. Each point represents the average values obtained from two animals.

appeared to be unaffected. In both control and harmine-pretreated animals the inhibition induced by SKF-385 followed approximately the same time course, suggesting that harmine in this dose did not interfere with the inhibitory action of SKF-385.

DISCUSSION

The results obtained in these experiments indicate that it is possible to protect the enzyme MAO from the long-acting inhibitory effects of PIH by first treating the animal with harmine, a short-acting reversible inhibitor. Similar indirect evidence also has been presented by Pletscher *et al*,[7,8] who found that the rise in brain levels of serotonin and norepinephrine resulting from the administration of iproniazid or glutamic acid-α-isopropylhydrazide could be reduced by prior treatment with harmaline. They attributed this effect to the inability of the hydrazine compounds to attack MAO which had been pretreated with harmaline, a circumstance which prevented the accumulation of the substrates of MAO in the brain. The present study confirms this hypothesis by direct examination of the enzyme system and has also demonstrated that even the extremely potent hydrazine derivatives, such as PIH, are effectively antagonized by pretreatment with harmine.

It is possible that the antagonism described here is the result of both drugs acting at the same site on the enzyme surface, and that the prior treatment with harmine prevents the occupation of the active site by PIH. A similar hypothesis has been offered to explain the antagonism of the DFP effects by physostigmine.[3] This phenomenon is seen only when the harmine is administered before the irreversible agent. If PIH be given before the shorter acting harmine, then the antagonism is no longer present, but the usual long acting effects of the hydrazines are produced. It would appear, therefore, that once the enzyme is irreversibly inhibited by PIH, harmine is no longer capable of attaching to the active site of the enzyme. This seems likely, since the inhibition of MAO by the hydrazines is irreversible,[1,9] while that produced by harmine is reversed quite readily.[10]

The inability of harmine-pretreatment to antagonize the long acting MAO-blocking actions of SKF-385, in the same manner as with PIH, suggests that its mechanism of action might differ from that of the hydrazine derivatives. No actual reports have appeared to indicate whether SKF-385 is a reversible or irreversible inhibitor, although structurally it resembles the reversible type of agent, such as amphetamine and ephedrine. Its relatively prolonged action, as demonstrated in these experiments, more closely resembles the irreversible type of inhibitor. Which ever the case may be, it appears that SKF-385 may be displacing harmine and itself occupying the active site. This is a possibility since some antagonism of the effect of SKF-385 was seen in brains of animals pretreated with very high doses of harmine. Thus, SKF-385 appears to have a very high affinity for the MAO enzyme. Some of these problems are under investigation at the present time.

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