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Comparison of Monoamine Oxidase Inhibitory Effects of Iproniazid and Its Phenyl Congener.* (24438)

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Iproniazid (1-isonicotinyl-2-isopropylhydrazine) has gained widespread interest in studies of the metabolism of biological amines. Its ability to inhibit monoamine oxidase (MAO) has greatly facilitated the understanding of the properties of this enzyme as well as of pharmacological and biochemical effects of serotonin (5-hydroxytryptamine) and norepinephrine in the brain. The MAO inhibitory properties of iproniazid *in vivo* were earlier described by Zeller *et al.*(1) and Schayer(2). Iproniazid has also been found to be effective in treatment of depressed mental patients. Its use in these cases presumably depends upon inhibition of MAO and prevention of the degradation of endogenous amines of the brain. These and other findings have stimulated the search for newer and more potent inhibitors of MAO. Recently the compound beta-phenylisopropylhydrazine† (PIH) was found to be some 30-50 times as potent as iproniazid in inhibiting MAO both *in vitro* and *in vivo*(3). The present report describes the effect of a closely related compound, 1-isonicotinyl-2-phenylisopropylhydrazine dihydrochloride‡ (isonicotinyl-PIH) on MAO activity. Isonicotinyl-PIH also resembles iproniazid, differing only by the presence of a phenyl group on the isopropyl chain. The similarities in structure of the 3 compounds are seen in Fig. 1.

Methods. Thirty-six male Wistar rats were used. *In vitro* MAO activity was determined by the method described by Sjoerdsma *et al.* (4) and by Bogdanski *et al.*(5). One milliliter of liver homogenate, 200 mg/ml was incubated with 4 μM of serotonin creatinine sulfate at pH 7.0. The inhibitors were added to the enzyme preparations and incubated 15 minutes prior to addition of substrate. Total volume of the incubation mixture was 3 ml. All incubations were made at 38°C in a constant temperature shaker. After incubation for 30 minutes the mixtures were extracted and assayed for residual serotonin according to the nitrosonaphthol method of Udenfriend, *et al.*(6). Readings were made on a Beckman DU spectrophotometer. *In vitro* studies were carried out using concentrations up to 10^{-4} M of the inhibitors. The effect of iproniazid and isonicotinyl-PIH on liver and brain MAO *in vivo* was also investigated. A dose of 5×10^{-5} M/kg of inhibitor was injected subcutaneously 2 hours prior to sacrifice of the animals. Tissues were quickly removed and homogenized and diluted to a concentration of 20% (liver) and 33% (brain). One

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† Beta-phenylisopropylhydrazine is designated as Compound JB-516, Lakeside Labs.

‡ The compound 1-isonicotinyl-2-phenylisopropylhydrazine dihydrochloride (isonicotinyl-PIH) was furnished for these studies by Lakeside Labs., under the designation of JB-821. Iproniazid was obtained through the courtesy of Dr. R. S. Floody, Hoffmann-LaRoche, Nutley, N. J.

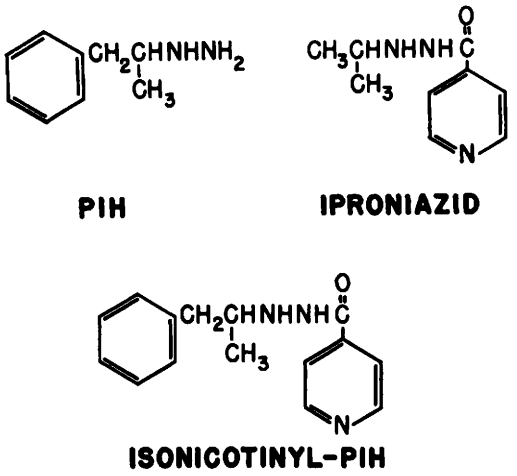

FIG. 1.

milliliter of the homogenate was incubated with the serotonin as described in the *in vitro* studies. Incubations with brains were carried out for 1 hour. The degree of MAO inhibition was calculated on the ability of the drug to decrease the rate of serotonin breakdown under the conditions employed. Results are graphed as % serotonin metabolized by the homogenates in the absence and presence of inhibitor.

Results. The results of *in vitro* inhibition of liver MAO by iproniazid and isonicotinyl-PIH are summarized in Fig. 2. Control homogenates metabolized approximately 70% of serotonin present during 30 minutes of incubation. In the presence of 10^{-4} M inhibitor, both iproniazid and isonicotinyl-PIH showed complete inhibition. At 5×10^{-5} M, inhibition by isonicotinyl-PIH was still essentially complete while the iproniazid effect had weakened and permitted about a 35% breakdown of substrate. When the concentrations of inhibitors were decreased to 10^{-5} M, serotonin breakdown occurred in both cases, but some inhibition was still present. Inhibition produced by iproniazid at this concentration was approximately 20% while isonicotinyl-PIH still exerted a 40% block. At both 5×10^{-5} and 10^{-5} M the difference in degree of MAO inhibition exerted by the 2 drugs was highly significant ($P<0.01$).

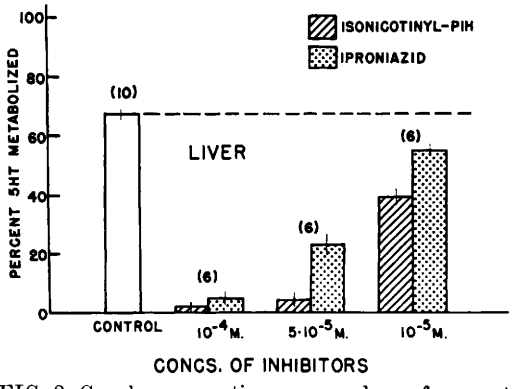

FIG. 2. Graph representing mean values of percent serotonin metabolized by rat liver homogenate in absence and presence of various concentrations of inhibitors. Vertical lines represent stand. error of mean values. Figures in parentheses indicate No. of experiments in each group.

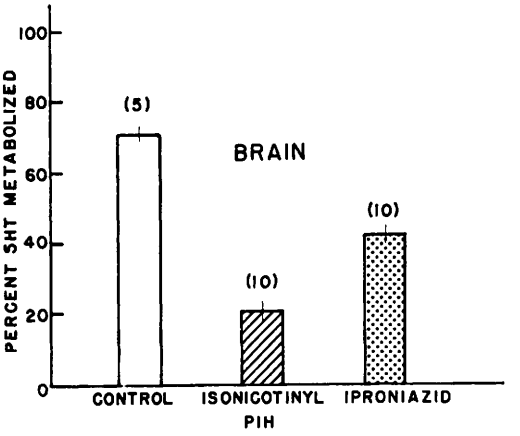

FIG. 3. Graph representing mean values of percent serotonin metabolism by brain homogenates prepared from control rats and rats pretreated with 5×10^{-5} M/kg of iproniazid and of isonicotinyl-PIH. Vertical lines represent stand. error of means. Figures in parentheses indicate No. of animals used in each group.

Liver and brain homogenates prepared from rats pretreated with the inhibitors showed results similar to the *in vitro* studies. At doses of $2\text{-}3 \times 10^{-5}$ M/kg, liver MAO was completely inactivated whereas the brain enzyme was still active. The dose of 5×10^{-5} M/kg was selected since both inhibitors produced sufficient inhibition to allow comparison of their relative effectiveness against brain MAO. Fig. 3 indicates activities of brain homogenates from control and inhibitor-treated rats in metabolizing serotonin. Isonicotinyl-PIH is approximately twice as effective as iproniazid in these preparations, showing a 70% inhibition. As in the *in vitro* results the dif-

ference in degree of inhibition by the 2 drugs was highly significant ($P<0.001$).

Discussion. The results of these experiments indicate that addition of a phenyl grouping to the isopropyl chain in iproniazid results in a compound with greater MAO inhibiting properties. Comparing the results obtained earlier with beta-phenylisopropylhydrazine (PIH)(3) it is also evident that the addition of an isonicotinyl group to the terminal N greatly decreases this property. This agrees with the studies of Davison(7) in which isopropylhydrazine was shown to be a much more potent MAO inhibitor than its isonicotinyl derivative (iproniazid). Although the effectiveness of MAO inhibition is reduced by addition of the isonicotinyl group to PIH, some of the pharmacological effects of the latter compound are also reduced or eliminated (unpublished observations). Studies of these compounds thus far indicate that isonicotinyl-PIH resembles iproniazid in its MAO inhibitory actions as well as in its pharmacological properties more closely than PIH. Such factors must be taken into consideration in the event that these compounds become useful therapeutic agents.

Summary. Comparison of monoamine oxidase (MAO) inhibiting properties of iproniazid and its phenyl congener, 1-isonicotinyl-2-phenylisopropylhydrazine (isonicotinyl-PIH) showed it to be 1.5 to 2 times as effective in inhibiting MAO both *in vitro* and *in vivo*. The results indicate that the addition of a phenyl group to the isopropyl chain of iproniazid enhances MAO inhibitory properties. Comparisons with beta-phenyl-isopropylhydrazine also indicate that the isonicotinyl group hinders the MAO inhibiting property of the former compound.

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