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THE ANTAGONISM OF HYDRAZINE-TYPE INHIBITORS OF MONOAMINE OXIDASE BY SODIUM PYRUVATE

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THE ANTAGONISM OF HYDRAZINE-TYPE INHIBITORS OF MONOAMINE OXIDASE BY SODIUM PYRUVATE

The influence of various metabolic intermediates and cofactors on the oxidative deamination of amines by monoamine oxidase (MAO) has been studied quite extensively (Govier et al, 1946; Wiseman and Sourkes, 1961; Meltzer, 1961). Govier et al (1946) observed that pyruvic acid enhanced the oxidation of tyramine and postulated that this was possibly the result of a coupled reaction between MAO and the cytochrome - cytochrome oxidase systems. The recent studies of Gey and Pletscher (1961) demonstrated a rise in blood pyruvic and lactic acids in rats after treatment with MAO inhibitors. The authors suggested that these increases were probably related to an alteration in monoamine metabolism, since injection of the amines also produced increases in both blood pyruvic and lactic acids.

Some of our recent work has dealt with the influence of pH on the metabolism of 5-hydroxytryptamine (serotonin, 5HT) by rat tissue preparations. In these studies, evidence was presented that in addition to MAO, a second enzyme, presumably the cytochrome oxidase system, was present in certain tissues and was capable of metabolizing 5HT when incubated at higher pH level (Horita, 1962). During these investigations certain of the metabolites of the tricarboxylic acid cycle were also included in order to determine their influence on 5HT metabolism. While most of the metabolites demonstrated no unusual effects, the addition of Na pyruvate to incubation mixtures containin g iproniazid or β-phenylisopropylhydrazine (PIH, JB-516) as MAO inhibitors produced unexpected results. Normally, the inhibitors were of sufficient

concentration to exert complete inhibition of MAO, but in the presence of pyruvate, the enzyme continued to metabolize the substrate to an almost equal degree as in the absence of inhibitor. The present study was therefore undertaken to determine the nature of this unusual phenomenon.

Method

Male Sprague-Dawley rats weighing 200 - 300 gms were used for both in vitro and in vivo enzyme experiments. MAO activity was measured on rat tissue homogenates by the method of Udenfriend et al (1958). 5HT (6 µmoles) was incubated with 1 ml of 20 percent liver or 33 percent brain homogenate in phosphate buffer at pH 7.4. After 30 and 60 minutes, respectively, aliquots of the mixtures were measured for residual 5HT by the colorimetric method employing the nitrosonaphthol - nitrous acid reagents. In vivo inhibition of MAO was determined by administering the designated drugs intraperitoneally to rats. The animals were sacrificed after two hours and the brains and livers analyzed for MAO activity as described above. In most instances where pyruvate was followed by the MAO inhibitor, not more than 5 minutes elapsed between the injections of the two drugs.

The blood pressure effects of PIH were studied on anesthetized dogs. A carotid artery was connected by a polyethylene tube to a Statham pressure transducer, and tracings of the responses were made on a Gilson Minipolygraph. The animals were anesthetized with 30 mg/kg of pentobarbital Na. All injections were made intravenously by means of a polyethylene cannula inserted into a femoral vein.

Results

Control preparations of rat liver homogenates metabolize 5HT at a linear rate for at least 30 minutes. The addition of sodium pyruvate (final conc., 0.05 - .1M) decreases this rate only slightly. Concentrations of 10^{-5} M PIH, however, are normally effective in completely inhibiting MAO, and 5HT disappearance (an index of MAO activity) is absent. If, however, the PIH is added to homogenates containing pyruvate, the normal inhibition of the enzyme does not occur, and 5HT breakdown proceeds at a rate between control and inhibited preparations. This apparent antagonism is even greater if the pyruvate and PIH are mixed prior to addition to the incubation mixture (Fig. 1). Progressively increasing the concentration of PIH will lead to complete inhibition of the enzyme. Also, if the PIH is added to the enzyme preparation before the pyruvate and permitted to exert its inhibitory action, then the addition of pyruvate does not reverse the inhibition.

A number of other well known hydrazine-type inhibitors of MAO were also examined for their ability to interact with pyruvate. As indicated in Table 1 the MAO inhibiting activity of all compounds with the -NH-NH- or $-NH-NH_2$ structures were antagonized to some extent. Other non-hydrazine inhibitors of MAO, such as tranylcypromine (SKF-385, 2-phenylcyclopropylamine), N-benzyl-N-methyl-2-propynylamine (MO-911), and harmine, were not affected in their property of inhibiting MAO by preincubation or simultaneous incubation with sodium pyruvate.

In the intact rat similar observations were made when PIH was used as the inhibitor. Animals pretreated with 500 mg/kg of sodium pyruvate and followed immediately with 1 mg/kg of PIH demonstrated little inhibition of both brain and liver MAO activity. Normally, this dose of PIH is capable of inhibiting the liver and brain to about 70 to 80 per cent. Iproniazid, however, did not demonstrate in vivo the consistent results as shown with PIH. The inhibition of MAO as produced by iproniazid was not antagonized by pretreatment of the animal with sodium pyruvate. The pre-incubation of the inhibitor with pyruvate before injection was also ineffective in preventing the action of iproniazid (Fig. 2). Several other hydrazine-type inhibitors of MAO were also examined for antagonism in vivo by pyruvate. Phenelzine was somewhat similar to PIH in its ability to become inactivated, but isocarboxazid and nialamide did not demonstrate loss of activity in the presence of sodium pyruvate in vivo. The non-hydrazine compounds were also unaffected by pyruvate in the intact animal.

While the antagonism of the enzyme effects of some of these hydrazines could be readily demonstrated, no indication of an alteration of the pharmacological actions could be determined by these procedures. We therefore turned to the cardiovascular actions of PIH in order to determine whether pyruvate antagonism could be demonstrated. The intravenous administration of PIH to anesthetized dogs or cats consistently leads to a pressor response (Eltherington and Horita, 1960). In lower doses (1 mg/kg) responses of equal intensity may be reproduced with repeated doses, but larger amounts (5 mg/kg) demonstrate an amphetamine-like tachyphylaxis. When equimolar concentrations of PIH and sodium pyruvate are mixed in a test tube, then injected into a dog,

the usual pressor response is absent. Very little effect on blood pressure is noted, and even with larger doses, the responses are minimal. After several such injections of high doses of the PIH-pyruvate mixture, PIH alone is still responsive in exerting its typical long-acting pressor response. Normally, the tachyphylactic condition is apparent after a single dose of 5 mg/kg of PIH.

In other similar experiments the animals were pretreated with large doses of sodium pyruvate and then followed with PIH. Unlike the PIH-pyruvate solution which had been preincubated before injection, PIH produced a pressor response of approximately equal magnitude as with control injections. The sodium pyruvate doses were raised as high as 500 mg/kg, but even this dose did not significantly alter the pressor response to PIH.

Discussion

The present study has demonstrated the ability of sodium pyruvate to antagonize the biochemical and pharmacological actions of several of the MAO-inhibiting hydrazine compounds. This antagonism appears to be specific for the hydrazine-type inhibitors, for the actions of non-hydrazine inhibitors such as harmine, SKF-385, and MO-911, were unaffected. The nature of this phenomenon appears to involve a chemical reaction between the hydrazine compounds and sodium pyruvate to form a hydrazone. A similar type of reaction was reported by Barreto and Mano (1961) in whose study the toxic actions of isoniazid were abolished by pretreating rabbits with sodium pyruvate. These authors attributed this change in toxicity to the formation of a hydrazone between isoniazid and pyruvic acid. Such a reaction was earlier demonstrated to occur *in vitro* by Lisboa (1959).

The formation of a hydrazone presumably involves the reaction between the terminal -N- of the hydrazine grouping and the carbonyl -C= of pyruvate, resulting in a -N-N=C- type of bonding. In order for such a reaction to occur a primary hydrazine ($-NH-NH_2$) would be required. Yet, compounds such as iproniazid, nialamide, and isocarboxazid, all of which are secondary hydrazines, are also affected by pyruvate in their ability to inhibit MAO in vitro. This discrepancy may be explained on the basis of a possible splitting of a substituted hydrazine molecule to yield a primary hydrazine which could react with the pyruvate. That such a mechanism is involved in the inhibition of MAO by substituted hydrazines has been suggested by Davison (1957), by Weikel and Salmon (1962), and by Schwartz (1961, 1962). This may also explain the lack of antagonism in vivo of the iproniazid-induced inhibition of MAO by pyruvate. Sufficient pyruvate may not be present at the site of liberation of the unsubstituted hydrazine moiety; consequently, inactivation of the molecule through hydrazone formation does not occur before enzyme inhibition is accomplished.

In addition to its property of inhibiting MAO, PIH possesses amphetamine-like actions on the cardiovascular system (Eltherington and Horita, 1960). This action is also abolished upon incubating the drug with pyruvate before its administration. This was expected, for if the hydrazone is formed, the structure of PIH would no longer be optimal for sympathomimetic activity. An important indication for the alteration of the PIH molecule when mixed with pyruvate is the gradual development of a fine suspension upon standing. Apparently, this readily formed substance is less soluble than the original compounds, thus tending to settle out as a fine precipitate.

Pretreating the animal with large doses of pyruvate, however, did not antagonize the pressor response to PIH. Probably the cardiovascular action of PIH is similar to that of amphetamine in that it reacts rapidly with the receptor site. This is unlike the enzyme effect which requires a greater period of time for complete inhibition to occur.

The significance of the inactivation of the hydrazine-type inhibitors of MAO by pyruvic acid may not be immediately apparent. The rapidity of this reaction suggests that part of the pathway of detoxication of the unsubstituted hydrazines may be through the formation of such hydrazones. Indeed, the work of Zamboni and Defranceschi (1954) demonstrate the formation of isonicotinylhydrazones of pyruvic and α-ketoglutaric acids in the urines of rats pretreated with isoniazid. Preliminary work in this laboratory also indicates α-ketoglutarate, dl-glyceraldehyde, and other carbonyl- containing compounds to be effective antagonists of the MAO-inhibiting hydrazines. Considering the number and concentration of such aldehydes and ketones in tissues, it raises the possibility of their being involved in the formation of hydrazones after administration of these inhibitors. Perhaps some of the toxic effects of the hydrazines may be the result of important carbonyl-containing metabolites being attacked, or through the formation of toxic hydrazone structures. Such a mechanism has been currently considered by McCormick and Snell (1959) and by Dubnick et al (1960) as explanations for the increased toxicity of the convulsant hydrazines after interaction with pyridoxal phosphate, a coenzyme involved in numerous important biochemical reactions.

SUMMARY

The incubation of several hydrazine-type inhibitors of monoamine oxidase (MAO) with 0.05 - .10 M sodium pyruvate resulted in a considerable loss of enzyme inhibiting activity. Non-hydrazine inhibitors of MAO were unaffected by this procedure. The activity of the unsubstituted compound, β-phenylisopropylhydrazine (PIH) was also antagonized when injected after administration of large doses of sodium pyruvate to rats. Substituted hydrazines, however, were not antagonized under these in vivo conditions. The preincubation of PIH with pyruvate also caused a loss of pressor activity of the hydrazine compound.

It is concluded that the antagonism of the biochemical and pharmacological actions of the hydrazine-type inhibitors of MAO by sodium pyruvate results from an interaction of the hydrazine moiety with the carbonyl group of pyruvate, leading to the formation of a biologically inactive hydrazone structure.

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TABLE 1

INFLUENCE OF SODIUM PYRUVATE ON INHIBITION OF RAT LIVER MAO BY VARIOUS COMPOUNDS

DRUG	MOLAR CONCENTRATION	PER CENT INHIBITION	
		Control	Pyruvate
Iproniazid	10^{-3}	100	67
PIH	10^{-5}	100	49
Phenelzine	5×10^{-5}	99	64
Isocarboxazid	2×10^{-5}	100	55
Nialamide	10^{-4}	81	38
MO-911	5×10^{-6}	80	95
SKF-385	10^{-5}	100	100
Harmine	10^{-5}	90	90

Each value of per cent inhibition represents the mean of triplicate determinations. In the column under "pyruvate" the incubation mixture contained 0.1 M Na pyruvate which was added 5 minutes prior to the addition of the inhibitors.

LEGENDS FOR FIGURES

Fig. 1. Graph representing the metabolism of 5HT by rat liver homogenates under the following conditions: (1) control; (2) presence of 0.1 M Na pyruvate; (3) addition of mixture of PIH (10^{-5} M) and Na pyruvate, preincubated before addition to enzyme preparation; (4) PIH (10^{-5} M) added to enzyme preparation containing pyruvate; and (5) addition of PIH alone. Only the preparation to which PIH alone was added exhibited complete inhibition of 5HT metabolism. Each point represents the mean of 3 to 5 determinations.

Fig. 2. Bar graph representing per cent inhibition of MAO activity of liver and brain homogenates prepared from rats pretreated with Na pyruvate, PIH, iproniazid (IIH), or pyruvate + inhibitor. Dosage of Na pyruvate, 500 mg/kg; PIH, 1 mg/kg; iproniazid - for liver MAO inhibition, 2 mg/kg, for brain MAO inhibition, 20 mg/kg. Each bar represents the mean values obtained from 6 animals. Vertical lines indicate standard deviation of the means.

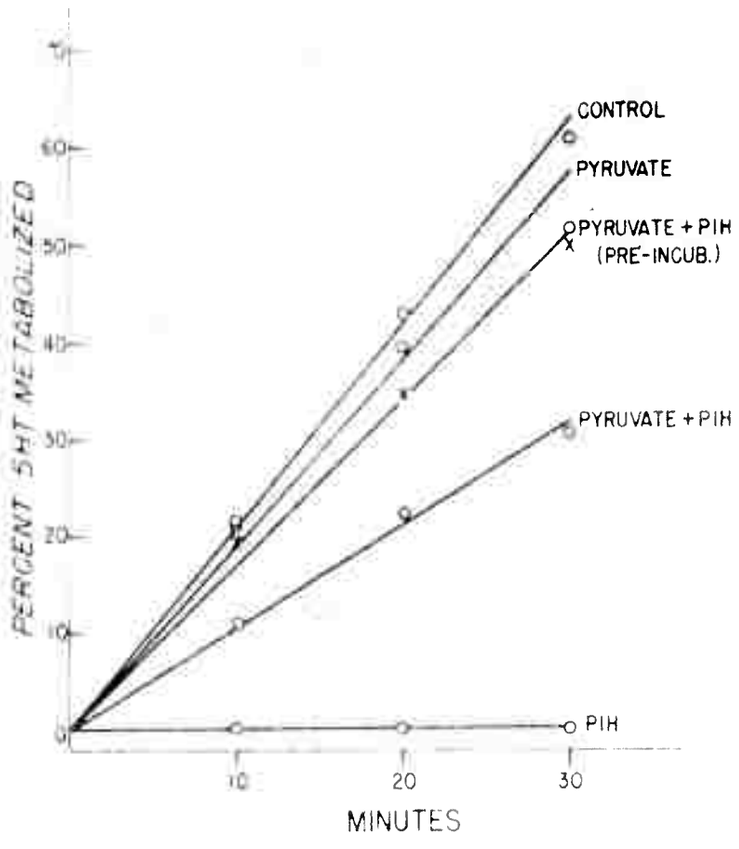
PERCENT 5HT METABOLIZED
0
10
20
30
40
50
60
CONTROL
PYRUVATE
PYRUVATE + PIH
(PRE-INCUB.)
PYRUVATE + PIH
PIH
10
20
30
MINUTES

Fig. 1

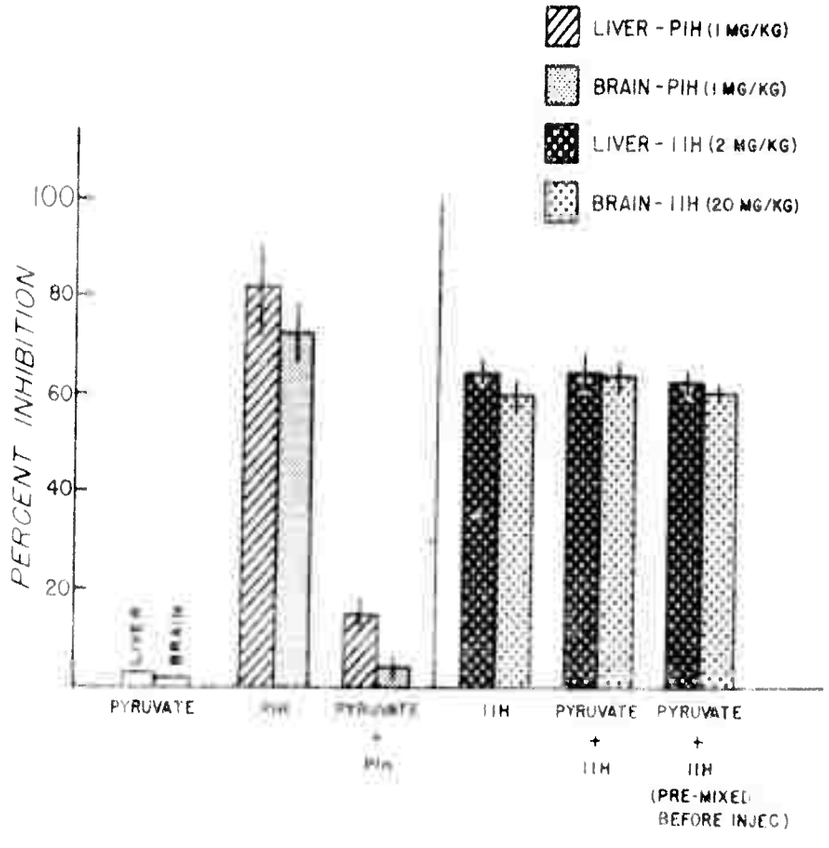
LIVER - PIH (1 MG/KG)
BRAIN - PIH (1 MG/KG)
LIVER - IIH (2 MG/KG)
BRAIN - IIH (20 MG/KG)
PERCENT INHIBITION
100
80
60
40
20
LIVER
BRAIN
PYRUVATE
PIH
PYRUVATE + PIH
IIH
PYRUVATE + IIH
PYRUVATE + IIH (PRE-MIXED BEFORE INJEC.)

Fig. 2

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