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EFFECTS OF 1-(INDOLYL-2-CARBONYL)-2-ALKYL-HYDRAZINE DERIVATIVES ON AMINE LEVELS AND MONOAMINE OXIDASE ACTIVITY IN RAT TISSUES

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Abstract—The inhibitory effects of five new derivatives of 1-(indolyl-2-carbonyl)-hydrazine on the monoamine oxidase activity in the rat brain and liver have been studied *in vivo* and compared with those of iproniazid.

Among the compounds investigated 1-(indolyl-2-carbonyl)-2-ethyl hydrazine (I) and 1-(indolyl-2-carbonyl)-2-isopropyl hydrazine (II), administered intraperitoneally to rats, inhibited the monoamine oxidase activity in the brain to the same extent of iproniazid. The inhibitory effects of the former compounds were delayed with respect to the latter but lasted longer.

Compound I caused an increase in the levels of 5-hydroxytryptamine and catecholamines in the rat brain similar to that produced by iproniazid and larger than that observed for compound II. Compound II was more effective than compound I in raising the levels of the heart catecholamines but the increase observed was smaller than that produced by iproniazid.

The new compounds studied were all less toxic than iproniazid when given orally or intraperitoneally to rats.

ZELLER *et al.*,[1] have shown that iproniazid (1-isonicotin-2-isopropylhydrazine) was a powerful inhibitor of the enzyme monoamine oxidase (MAO). Since then, it has been repeatedly demonstrated that many compounds having an antidepressive or psycostimulant action have the capacity of inhibiting the activity of this enzyme.[2,3] In addition it has been shown that some MAO inhibitors possess hypotensive effects and for this reason these compounds have been used in the treatment of angina pectoris and hypertension.[4,5] The clinical use of hydrazine derivatives, however, has been limited by their toxicity on the liver cells and other deleterious side-effects.[6–8]

These findings have stimulated, in recent years, the search for new compounds that would combine a high MAO inhibiting activity with a low liver toxicity.

Alemany *et al.*,[9] have shown that some 2-alkyl derivatives of 1-(indolyl-2-carbonyl)-hydrazine possessed marked inhibitory effects on the activity of MAO *in vitro*.

The molecule of these compounds has an indole nucleus, which is believed to play an important role in some processes of the central nervous system,[10] and an hydrazine group whose psycostimulant properties are well known.[11]

The purpose of this study was to investigate, *in vivo*, the effects of this new type of compounds on the MAO activity in rat liver and brain. In addition the levels of catecholamines in the brain and heart and the levels of 5-hydroxytryptamine in the brain of rats treated with these new MAO inhibitors have been also determined. Finally, the toxicity of the compounds under investigation has been compared with that of iproniazid.

MATERIALS AND METHODS

Wistar rats weighing 150–200 g were used. Compounds were injected intraperitoneally as 1 per cent suspension in arabic gum. The rats were sacrificed by decapitation and the organs to be studied were quickly removed, weighed and homogenized in the cold.

The new synthetic inhibitors studied were; (I), 1-(indolyl-2-carbonyl)-2-ethylhydrazine; (II), 1-(indolyl-2-carbonyl)-2-isopropylhydrazine; (III), 1-(indolyl-2-carbonyl)-2-sec-butylhydrazine; (IV), 1-(indolyl-2-carbonyl)-2-(1-ethyl-1-propyl)hydrazine; (V), 1-(indolyl-2-carbonyl)-2-(1-methyl-3-phenylethyl)hydrazine.

The toxicity of the compounds was evaluated by orally and intraperitoneally administering doses from 100 to 500 mg/kg of each inhibitor to the rats. The rats were kept under observation for a period of 4 days.

The inhibition of MAO in the tissues was determined by the method of Ozaky *et al.*[12] 5-Hydroxytryptamine in the brain was measured by the method of Mead and Finger.[13] The brain and heart catecholamines were assayed by the procedure of Shore and Olin,[14] as modified by Callingham and Cass.[15] By this method adrenaline and noradrenaline are measured together and expressed in terms of noradrenaline.

RESULTS AND DISCUSSION

The inhibitory effects of compounds I, II, III, IV and V on the MAO activity, *in vivo*, were studied in two series of experiments. In the first series, 100 mg/kg of the compounds mentioned above and iproniazid were administered intraperitoneally to six groups of five rats each. Control animals received an equivalent amount of vehicle. After 24 hr the animals were killed and the MAO activity in liver and brain was determined.

TABLE 1. INHIBITION *in vivo* OF MAO ACTIVITY IN RAT TISSUES BY IPRONIAZID AND 2-ALKYL DERIVATIVES OF 1-(INDOLYL-2-CARBONYL)-HYDRAZINE

Compound	Inhibition (% of control)		
	Liver	Brain	Liver/brain
Iproniazid	97 ± 0·4	92 ± 3·6	1·05
I	87 ± 5·5	82 ± 4·6	1·06
II	86 ± 6·4	76 ± 5·0	1·13
III	64 ± 9·0	37 ± 14·5	1·73
IV	85 ± 1·4	51 ± 11·5	1·67
V	76 ± 8·5	29 ± 13·7	2·62

Rats received the test compounds (100 mg/kg, i.p.) and were killed 24 hr after the injection. There were five animals in each treated group and 15 in the control.
Values are means ±S. E.

Table 1 shows the percentage inhibition obtained with each of the compounds tested. It may be seen that there is no significant difference between the inhibition of MAO activity in the liver and brain of animals treated with iproniazid and compound I.

Iproniazid and compound II produced a similar inhibition of the MAO activity in the liver but compound II inhibited the enzyme to a lesser extent than iproniazid in the brain.

All the other compounds tested inhibited MAO activity less efficiently than iproniazid.

One of the aims of the present investigation was to find out whether any of the new inhibitors studied had a preferential action on the MAO activity in the brain. Table 1, shows that the ratio of the inhibitions observed in the liver and brain for compounds I and II was close to 1·0 and similar to that found for iproniazid. Compounds III, IV and V inhibited preferentially the MAO activity in the liver.

In the second series of experiments three groups of twenty rats were given 100 mg/kg of compounds I, II and iproniazid intraperitoneally. Each group was then divided into four lots of five animals each. Four groups of five untreated animals were included as controls. The animals were sacrificed 2, 24, 48 hr and 8 days after the injection of the inhibitor and the activity of the MAO in the brain and liver was measured. Table 2, summarizes the results obtained. It may be seen that the inhibition of the

TABLE 2. INHIBITION *in vivo* OF MAO ACTIVITY IN RAT TISSUES AFTER ADMINISTRATION OF IPRONIAZID AND COMPOUNDS I AND II, EFFECT OF TIME

	Inhibition (% of control)					
	Brain			Liver		
Compound	Iproniazid	I	II	Iproniazid	I	II
Time after injection						
2 hr	78 ± 3·6	9 ± 6·6	33 ± 10·0	87 ± 3·6	78 ± 5·1	86 ± 3·3
24 hr	92 ± 3·6	82 ± 4·6	76 ± 5·0	97 ± 0·4	87 ± 5·5	86 ± 6·4
48 hr	78 ± 3·9	91 ± 9·0	71 ± 9·4	79 ± 5·3	80 ± 9·0	89 ± 7·4
8 days	45 ± 3·4	62 ± 3·8	55 ± 8·8	39 ± 5·6	66 ± 5·6	73 ± 9·8

Rats were injected with 100 mg/kg (i.p.) of the test compounds. There were five animals in each treated group and 15 in the control.
Values are means ±S. E.

MAO activity observed in the animals killed 2, 24 and 48 hr after the administration of compounds I and II is similar to that observed in the liver of the animals treated with iproniazid. It is also apparent that the inhibitory effects of compounds I and II lasted longer than those of iproniazid, the compound taken as reference.

Table 2, also shows that the inhibition of the MAO activity in the brain of the rats killed 2 hr after the administration of compounds I and II is much less pronounced than that observed in the group of rats treated with iproniazid but that the inhibition produced by the three compounds in the brain of the animals sacrificed 24 and 48 hr after the beginning of the experiment were very similar.

In a third series of experiments the concentration of 5-hydroxytryptamine in the brain of rats treated with compounds I and II was compared with that of a group of animals treated with iproniazid. Drugs (100 mg/kg), were administered intraperitoneally to rats that were sacrificed 2, 24 or 48 hr after the injection. A group of four animals was used for each experiment. The control animals were given an amount of arabic gum equal to that administered to the animals receiving the drugs.

TABLE 3. 5-HYDROXYTRYPTAMINE LEVELS IN RAT BRAIN AFTER ADMINISTRATION OF IPRONIAZID AND COMPOUNDS I AND II

Compound	Iproniazid	I	II
Time after injection			
2 hr	0·74 ± 0·01	0·73 ± 0·10	0·56 ± 0·07
24 hr	1·08 ± 0·04	0·64 ± 0·06	0·48 ± 0·03
48 hr	1·23 ± 0·06	1·05 ± 0·04	0·98 ± 0·07

Rats were injected with 100 mg/kg (i.p.) of the test compounds. There were four animals in each treated group and 12 in the control. The mean value ±S. E. of 5-hydroxytryptamine concentration in the brain of the controls was 0·36 ± 0·04 μg/g.
Values (μg/g) are means ±S. E.

Table 3 shows the results obtained. It can be seen that the concentration of 5-hydroxytryptamine in the brain of the rats treated with the drugs under investigation was significantly higher than that of the control animals and that iproniazid induced a somewhat higher amine levels than compounds I and II.

In another series of experiments the concentration of catecholamines (adrenaline and noradrenaline) in the brain and heart of rats treated with iproniazid, compounds I and II were also determined. As in the previous series of experiments the animals were given the drugs under study (100 mg/kg),and were killed 2, 24 and 48 hr after the injection. A group of 18 control rats received only the vehicle.

TABLE 4. CATECHOLAMINES (ADRENALINE AND NORADRENALINE) LEVELS IN RAT BRAIN AND HEART AFTER ADMINISTRATION OF IPRONIAZID, COMPOUND I AND COMPOUND II

	Brain			Heart		
Compound	Iproniazid	I	II	Iproniazid	I	II
Time after injection						
2 hr	0·64 ± 0·04	0·62 ± 0·08	0·42 ± 0·05	1·59 ± 0·23	0·91 ± 0·09	1·20 ± 0·11
24 hr	0·83 ± 0·01	0·81 ± 0·10	0·54 ± 0·02	1·40 ± 0·05	1·10 ± 0·05	1·27 ± 0·15
48 hr	1·01 ± 0·04	0·77 ± 0·08	0·76 ± 0·07	0·97 ± 0·11	1·29 ± 0·12	1·17 ± 0·03

Rats received 100 mg/kg (i.p.) of the test compounds. There were four animals in each treated group and 18 in the control. The mean values ±S. E. of catecholamines concentration in the brain and heart of the controls were respectively 0·48 ± 0·04 μg/g and 1·08 ± 0·09 μg/g.

The concentrations of catecholamines (expressed as noradrenaline) in the brain are shown in Table 4. The variations in the concentration of these hormones induced by the three MAO inhibitors are less marked than those observed for 5-hydroxytryptamine. It can also be seen that the effect of compound II manifest itself later than that of compound I and that the increase in the concentration of the brain catecholamines induced by these two MAO inhibitors is smaller than that induced by iproniazid under identical experimental conditions. Table 4 shows also the concen-

tration of catecholamines in the rat heart after treatment of the animals with iproniazid, compound I and compound II. The drugs (100 mg/kg), were administered intraperitoneally and the animals were sacrificed 2, 24 or 48 hr after the injection. Of the three MAO inhibitors tested only iproniazid induced a significant increase of the concentration of catecholamines in the heart of the rats sacrificed 2 or 24 hr after treatment.

In one series of experiments doses of 100, 200, 300, 400 and 500 mg/kg of compounds I, II, III, IV and V and iproniazid were administered intraperitoneally to rats. Four or five animals were used to test the toxicity of each compound at each dose. In another series of experiments the same amount of inhibitors was given orally. All the animals that received compounds I, II, III, IV or V at any of the doses tested (up to 500 mg/kg) survived and during the 4 days of observation none showed symptoms of intoxication. On the other hand all the rats that had received iproniazid at concentrations above 300 mg/kg intraperitoneally or above 200 mg/kg orally died within 24 hr.

All the animals used for the evaluation of the toxicity of the new MAO inhibitors were sacrificed 4 days after the administration of the compounds. A post mortem examination of the viscerae did not show macroscopic alterations.

The present investigation has shown that two of the five compounds tested have MAO inhibiting properties *in vivo* comparable to those of the well known substance iproniazid. A time study of the inhibiting properties of compounds I and II on the MAO activity in the brain and liver of rats has revealed that the effects produced by the two compounds appears later but last longer than those caused by iproniazid.

Compounds I and II are also effective in increasing the concentrations of 5-hydroxytryptamine and catecholamines in the brain of rats but do not influence the concentrations of these amines in the heart of the same animals.

It has been found that the five compounds tested are all less toxic than iproniazid.

REFERENCES

1. E. A. Zeller, J. Barsky, J. R. Fonts, W. F. Kirchheimer and L. S. Van Order, *Experientia* **8**, 349 (1952).
2. J. Rebhum, S. M. Feinberg and E. A. Zeller, *Proc. Soc. exp. Biol. N.Y.* **87**, 218 (1954).
3. L. Gillespie, *Ann. N.Y. Acad. Sci.* **88**, 1011 (1960).
4. A. M. Master, *Am. Heart J.* **56**, 570 (1958).
5. M. H. Maxwell, *Ann. N.Y. Acad. Sci.* **107**, 993 (1963).
6. L. Zetzel and H. Kaplan, *Engl. J. Med.* **258**, 1209 (1958).
7. H. J. Zimmermann, L. Rosenblum, R. J. Korn and P. E. Feldman, *Ann. N.Y. Acad. Sci.* **80**, 915 (1959).
8. G. Zbinden and A. Studez, *Ann. N.Y. Acad. Sci.* **80**, 873 (1959).
9. A. Alemany, M. Bernabe, C. Elorriaga, E. Fernandez-Alvarez, M. Lora-Tomayo and O. Nieto, *Bull. Soc. Chim.* **8**, 2486 (1966).
10. C. L. Zirkle and C. Kaiser, *Psychopharmacological Agents*, Vol. I, pp. 445–554. Academic Press, London (1964).
11. J. H. Biel, A. Horita and A. E. Drukker, *Psychopharmacological Agents*, Vol. I, pp. 359–443. Academic Press, London (1964).
12. M. Ozaky, H. Weissbach, A. Ozaky, B. Witkop and S. Udenfriend, *J. med. pharm. Chem.* **2**, 591 (1960).
13. J. A. R. Mead and K. F. Finger, *Biochem. Pharmac.* **6**, 52 (1961).
14. P. A. Shore and J. S. Olin, *J. Pharmac. exp. Ther.* **122**, 295 (1958).
15. B. A. Callingham and R. Cass, *The clinical chemistry of monoamines*, pp. 19–28, Elsevier, Amsterdam (1963).