Biochemical Pharmacology, 1961, Vol. 8, pp. 263–268. Pergamon Press Ltd., Printed in Great Britain.

INVESTIGATIONS INTO THE CORRELATIONS BETWEEN MONOAMINE OXIDASE INHIBITION AND OTHER EFFECTS DUE TO METHYLPHENYDATE AND ITS STEREOISOMERS

L. SZPORNY and P. GÖRÖG

Pharmacological Laboratory, Chemical Works of Gedeon Richter Ltd., Budapest, Hungary

(*Received by Editors* 28 *February* 1961; *Received by Publishers* 29 *May* 1961)

Abstract—The inhibitory activity of methylphenydate and of threo- and erythro-methylphenydate in homogenates from liver, brain and kidney were investigated. This inhibition was also demonstrated *in vivo*. The monoamine oxidase inhibition produced is of similar strength in these two cases.

The acute hypertensive actions and toxicities of the threo and erythro isomers are of equal strength; but, in agreement with other work, only the threo derivatives display a locomotor stimulant action. No parallelism could be traced between the locomotor stimulant effect of stereoisomers and their inhibition of monoamine oxidase.

MONOAMINE oxidase is known to play an important role in the functions of the central nervous system. In recent years numerous papers have reported drugs that inhibit the function of this enzyme.[1–3] Some of them, e.g. iproniazid and its derivatives, as well as compounds that are not hydrazine derivatives,[4–6] have proved useful clinically, for the treatment of states of depression.

It is also known that amphetamine, which has proved itself an efficient stimulant for the central nervous system, is a potent monoamine oxidase inhibitor: this property is thought to be connected with its therapeutic value.

Methylphenydate has been found to be an effective stimulant of the central nervous system: it is weaker than amphetamine, but its use does not lead to addiction. This drug antagonizes the sedative action of reserpine without affecting its hypotensive effect.

This paper reports investigations on the action of methylphenydate on manoamine oxidase, with special reference to its stimulant effect on the central nervous system.

MATERIALS AND METHODS

We employed methylphenydate (Centedrin®, Richter Works) containing 80 per cent of erythro and 20 per cent of threo isomers. Studies have also been undertaken with the pure stereoisomers isolated by Weisz and Dudas.[7]

Monoamine oxidase inhibition was measured *in vitro* by the colorimetric method of Udenfriend[8] using serotonin (creatinine sulphate) substrate, as well as by manometry, again using serotonin as substrate.

Manometric determinations of monoamine oxidase activity in brain tissue were carried out *in vivo* in experiments on pretreated animals.

As sources of enzymes, we used homogenized rat liver and kidney (200 mg/ml), and brain (333 mg/ml).

Locomotor stimulant action was studied in albino mice for 30 min following intraperitoneal administration of the drug: a photoelectric apparatus was used for the measurement of motility.

Blood pressure was investigated in cats under intravenous urethane anaesthesis. Toxicity was studied by the method of Litchfield-Wilcoxon, after intravenous and intraperitoneal administration in albino mice.

RESULTS

The summarized results show that methylphenydate exerts marked inhibition on monoamine oxidase (Table 1). In high concentrations ($2{\cdot}8 \times 10^{-2}$ M) the drug

TABLE 1. INHIBITION OF MONOAMINE OXIDASE ACTIVITY IN RAT LIVER*

Drug	Final conc. (M)	Inhibition, measured by colorimeter (%)	Inhibition, measured by O_2 uptake (%)
Methylphenydate	$2{\cdot}8 \times 10^{-2}$	98	100
Methyphenydate	1×10^{-2}	47	44
Threo-methylphenydate		46	41
Erythro-methylphenydate		40	45
Methylphenydate	1×10^{-3}	26	30
Threo-methylphenydate		24	32
Erythro-methylphenydate		28	25
Methylphenydate	1×10^{-4}	13	17
Threo-methylphenydate		10	18
Erythro-methylphenydate		15	18
Amphetamine	1×10^{-2}	68	73
	1×10^{-3}	36	30
Iproniazid	5×10^{-3}	98	96
	1×10^{-3}	58	64

* Values are means of four experiments. Inhibition was measured after 30 min. The substrate was 0·01 M serotonin in both cases.

produced nearly 100 per cent inhibition of enzyme function. A concentration of 1×10^{-3} M was found to give almost 30 per cent inhibition. To provide a basis for comparison, the degree of inhibition at similar concentrations of amphetamine and iproniazid have also been included. The ratio of the monoamine oxidase inhibiting action of methylphenydate to that of amphetamine equals the ratio of their locomotor stimulant effects. The table also shows that the pure threo and erythro isomers are equally potent. The values obtained by the two methods are in agreement, and they exclude any possibility that the tested materials form enzyme substrates. The time required for inhibition is the same for the three compounds (see Fig. 1).

Since it is well known that inhibition of monoamine oxidase by various compounds may be different in different organs, the three isomers have also been studied in homogenized brain and kidney, as well as in homogenized liver. The values obtained

are shown in Fig. 2. The isomers gave the same inhibition of O_2 consumption, and their actions on the liver, brain and kidney were almost the same.

To determine the physiological significance of these experiments, we undertook *in vivo* experiments on rats. After pretreatment of the animals with intraperitoneal doses (50 mg/kg), cerebral monoamine oxidase activity was determined at intervals of $\frac{1}{2}$, 2, 6 and 16 hr later. The results obtained are presented in Fig. 3.

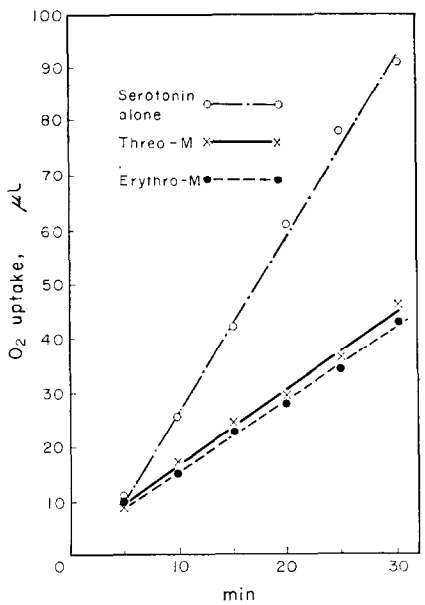

FIG. 1. Inhibition of monoamine oxidase in rat liver by stereoisomers of methylphenydate.

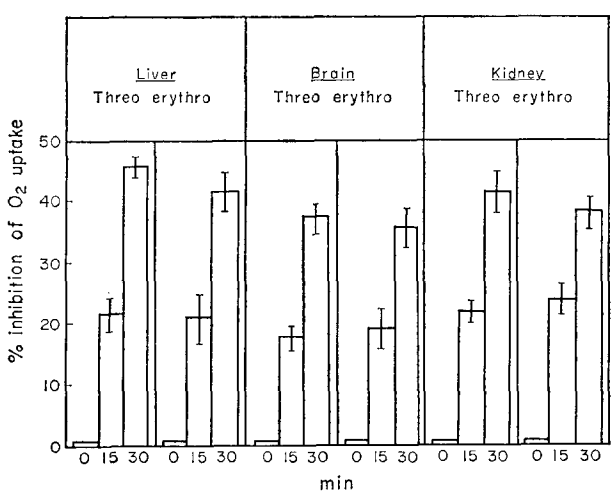

FIG. 2. Effect of methylphenydate stereoisomers on monoamine oxidase activity in rat brain, liver, and kidney homogenates. Vertical lines denote the standard errors of the assays.

As shown in the figure, monoamine oxidase is strongly inhibited by methylphenydate *in vivo* as well. The inhibitive effects of threo and erythro isomers, and the course and duration of the inhibition do not display any significant difference.

As the isomers exhibited an almost similar effect of monoamine oxidase under all experimental conditions, it appeared desirable to undertake comparative analysis of the same substances for locomotor stimulation.

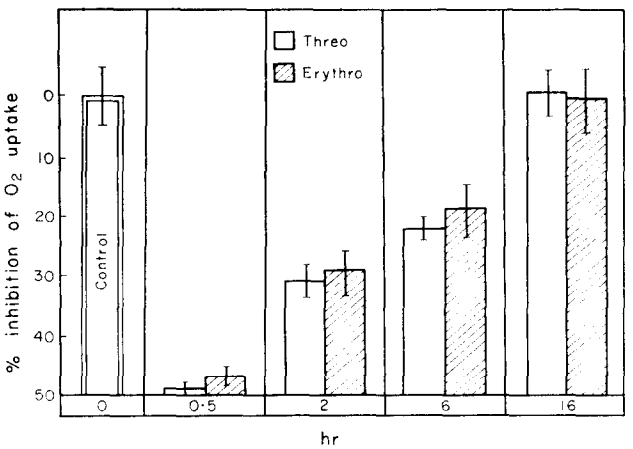

FIG. 3. *In vivo* investigation of the monoamine oxidase inhibitive activity of methylphenydate. The activity in the brain was measured at various times after the intraperitoneal injection of the drug in doses of 50 mg/kg.

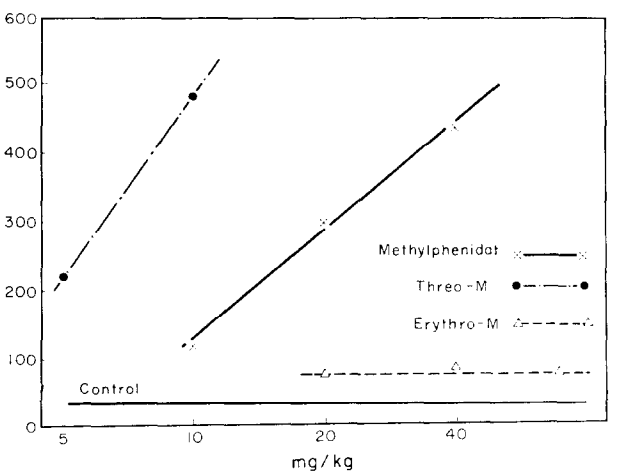

FIG. 4. Locomotor stimulating effect of methylphenydate and its stereoisomers on mice. The drugs were injected subcutaneously. Abscissa: doses (mg/kg); ordinate: number of disconnexions of light in 30 min, representing the rate of motility. Each point represents the mean value of at least ten mice.

Previous studies of this problem have shown that the threo isomer is the only effective locomotor stimulant.[9, 10] Confirmation of these results by a different method seemed to be justified. The results of our investigations are presented in Fig. 4, and the threo isomer is shown by these to be the only effective stimulant, while the erythro

isomer is ineffective. The mixture Centedrin owes its efficiency to the threo isomer which makes up 20 per cent of its contents.

Later we studied the immediate hypertensive action of the above compounds, and the results, presented in Fig. 5, show that there is no difference between the isomers.

Finally the three isomers were tested for acute toxicity: the values obtained are presented in Table 2. The LD_{50} values were essentially the same, whether administered intraperitoneally or intravenously.

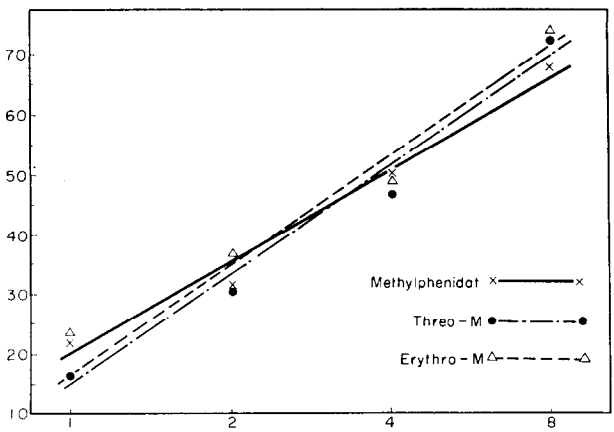

FIG. 5. Hypertensive activity of methylphenydate on cats in urethane narcosis. The ordinate represents the elevation of blood pressure as a percentage of its value before drug administration. The abscissa shows doses of the drugs (mg/kg) administered intravenously. Each point represents the mean for four animals treated with one single dose.

TABLE 2. TOXICITY VALUES OF METHYLPHENYDATE AND ITS STEREOISOMERS

Drug	LD_{50} (mg/kg injected intravenously)
Methylphenydate	41
Threo-methylphenydate	39
Erythro-methylphenydate	47

CONCLUSIONS

Methylphenydate exerts a marked inhibitive effect on monoamine oxidase under *in vivo* and *in vitro* conditions alike, as do other compounds having an anti-depressive effect. Only the threo isomer displays a locomotor stimulant effect, though all three isomers have an equal inhibiting effect on the enzyme. Only acute hypertensive action and acute toxicity of the isomers showed any parallelism with monoamine oxidase inhibition. Hence locomotor stimulant action is specific to stereo-structure, whereas the monoamine oxidase inhibition is not. Our experiments may be compared to the studies of Grana and Lilla,[11] who found that, when judged by locomotor stimulant action, *l*-amphetamine alone was active, while when tested for monoamine oxidase inhibition, the *l*- and *d*-isomers did not materially differ.

We therefore suppose that the locomotor stimulant action of these compounds is produced by a mechanism other than that responsible for the inhibition of monoamine oxidase.

Whether the anti-depressive action noted in connexion with methylphenydate and amphetamine therapy is due to the locomotor stimulant effect observed in animals or to the mechanism represented by the inhibition of monoamine oxidase, remains to be decided.

REFERENCES

1. E. A. ZELLER and J. BARSKY, *Proc. Soc. Exp. Biol., N.Y.* **81**, 459 (1952).
2. A. HORITA, *J. Pharmacol.* **122**, 176 (1958).
3. Symposium on Amine Oxidase Inhibitors, *Ann. N.Y. Acad. Sci.* **80**, 551 (1959).
4. K. FRETER, M. WEISSBACH, B. G. REDFIELD, S. UDENFRIEND and B. WITKOP, *J. Amer. Chem. Soc.* **80**, 983 (1958).
5. S. UDENFRIEND, B. WITKOP, B. G. REDFIELD and N. WEISSBACH, *Biochem. Pharmacol.* **1**, 160 (1958).
6. P. A. SHORE and V. H. COHN, *Biochem. Pharmacol.* **5**, 91 (1960).
7. I. WEISZ und A. DUDÁS, *Mh. Chem.* **91** (5), 840 (1960).
8. S. UDENFRIEND, H. WEISSBACH and C. T. CLARK, *J. Biol. Chem.* **215**, 337 (1955).
9. R. MEIER, F. GROSS und J. TRIPOD, *Klin. Wschr*, **32**, 445 (1954).
10. J. PORSZÁSZ. Personal communication.
11. M. GRANA and L. LILLA, *Brit. J. Pharmacol.*, **14**, 501 (1959).
12. P. PRATESI and H. BLASCHKO, *Brit. J. Pharmacol.* **14**, 261 (1959).