Biochemical Pharmacology, 1966, Vol. 15, pp. 1645–1647. Pergamon Press Ltd., Printed in Great Britain.

EDITORIAL

ASSESSMENT OF DRUG-INDUCED INHIBITION OF MONOAMINE OXIDASE ACTIVITY*

ROBERT J. LEVINE

Departments of Medicine and Pharmacology, Yale University School of Medicine, New Haven, Conn., U.S.A.

THIS essay is concerned with some problems that have been encountered in attempts to correlate inhibition of monoamine oxidase (MAO) activity in man with symptomatic and physiologic effects elicited by drugs that inhibit MAO activity *in vitro* (MAO inhibitors). Consideration of some of the goals of these studies may help bring the specific problems into focus. In one category, studies are designed to correlate MAO inhibition with a specific physiologic effect of a drug in order to define the mechanism by which the drug produces that effect. It is essential that these studies be done in man rather than in some experimental animal. In addition to the usual problems encountered with species variations, some of the most important effects of MAO inhibitors cannot be studied adequately in animals. For example, there are no completely acceptable animal models for the study of drug-induced postural hypotension or relief of angina pectoris or psychic depression. Once a temporal association between MAO inhibition and therapeutic effect has been established, it becomes useful to be able to measure some parameter of MAO inhibition frequently to provide a rational basis for adjustment of doses in individual patients. This is a second type of correlative study often undertaken.

Ideally, for the first type of study the technique used should provide a reliable indication of the degree of MAO inhibition in the specific tissue in which the drug produces its physiologic effect. Among the features desired for the technique employed in the second type of study are reliability under a variety of physiologic and environmental conditions and convenience for both the subject and the investigator.

In 1963 Levine and Sjoerdsma reviewed some of the techniques available at that time for study of MAO inhibition in man.[1] None of these methods is completely satisfactory. The most acceptable indirect technique consists of quantitative assay of urinary levels of amines such as tryptamine and tyramine that lack efficient alternative pathways for metabolism. Although indirect methods provide a satisfactory index of inhibition of total-body MAO activity, they yield no information with regard to inhibition of the enzyme in specific tissues such as brain and sympathetic nerve endings. Other problems are encountered occasionally in the application of these techniques. For example, variations in the dietary intake of protein or amino acids

* This editorial was solicited by the Regional Editor in the belief that it would be useful in guiding investigators in this area by pointing out pitfalls in research, of which earlier investigators may not have been cognizant.

may lead to significant changes in the urinary levels of the amines. Also, certain drugs and their metabolites produce extraneous interfering fluorescent substances. There is also available a direct method for assay of MAO activity in tissue.[1] For this purpose jejunal mucosa has been used because it has relatively high and reasonably constant levels of MAO activity, and because specimens may be obtained frequently without exposing subjects to undue risk or serious discomfort. Again, we have no assurance that inhibition of MAO activity in jejunal mucosa reflects accurately similar degrees of inhibition of the enzyme in other tissues. In spite of these problems it has been possible to demonstrate, by both indirect and direct methods, temporal correlations between inhibition of MAO activity and at least one therapeutic effect of MAO inhibitors; i.e. postural hypotension. These techniques have not proved entirely suitable for the purpose of monitoring dose requirements of MAO inhibitors. It is not ordinarily practical to require patients who are not hospitalized to obtain quantitative 24-hr collections of urine. Neither is it convenient to biopsy jejunal mucosa frequently in such patients.

In 1964 Otsuka and Kobayashi described in this journal[2] a new approach to monitoring MAO inhibition in man by assay of MAO activity in plasma. This method offered the important advantage of convenience to the subjects as well as to the investigator. However, there are some very important problems in interpreting the results of this approach.

There are at least three enzymes in human plasma that have amine oxidase activity; viz. ceruloplasmin, diamine oxidase, and monoamine oxidase. The conditions of assay described by Otsuka and Kobayashi indicate that they are not measuring either ceruloplasmin or diamine oxidase. Human plasma monoamine oxidase has been purified and studied in detail by McEwen.[3] He reports that it shows several important differences from the mitochondrial MAO found in tissue. To name a few: human plasma MAO is not associated with any particulate fraction as is tissue MAO. Substrates most actively oxidized by the plasma enzyme are simple aliphatic amines; some of the better substrates for the tissue enzyme, such as tyramine and tryptamine, are less actively oxidized. Beta-hydroxylated amines such as octopamine and norepinephrine are substrates for the tissue enzyme but not the plasma enzyme. Also, in contrast to the tissue enzyme, the plasma enzyme is inhibited by cuprizone and by carbonyl reagents such as semicarbazide.

In 1966 Kobayashi reported in this journal[4] studies of the effects of oral administration of various MAO inhibitors on human plasma MAO activity, determined by the technique Otsuka and he had described earlier. He stated that since the plasma MAO activity, as determined by his method, was inhibited only partially by semicarbazide, it was more similar to the tissue MAO than it was to the plasma enzyme characterized by McEwen. This would seem to imply that there is a fourth enzyme in human plasma with amine oxidase activity. Before we can accept assays of MAO activity in human plasma as a valid approach to monitoring therapy with MAO inhibitors, we must require rigorous proof that there exists in plasma an enzyme with MAO activity that is similar to that in tissue; that it is inhibited by the same substances that inhibit mitochondrial MAO; and that the method employed for its assay is specific for that enzyme. Perhaps in that regard it would be of value to use as substrate norepinephrine or octopamine or some other substance that is not a substrate for the enzyme characterized by McEwen.

As discussed by Kobayashi, there still exists no technique whereby we can correlate inhibition of MAO activity directly with therapeutic effects of MAO inhibitors in certain organs of specific interest such as brain. At this time there are only indirect methods by which we can infer that MAO activity has been inhibited in brain. For example, one may observe potentiation by MAO inhibitors of effects on the central nervous system of tryptophan loading.[5]

Finally, I should like to use this vehicle to make a general comment on enzyme assays that use radioactively-labeled substrates. We have seen recently in the pharmacologic literature the introduction of a number of assay techniques in which a labeled substrate is incubated with a crude tissue preparation for the purpose of determining enzyme activity. In these crude tissue preparations there are generally present unknown amounts of endogenous substrates, some of which may be even better substrates for the enzyme in question than the labeled one that is added to the incubation mixture. Since these dilute the specific activity of the added isotope to an unknown extent, it is impossible to derive from such assays quantitative determinations of enzyme activity. When these assays are applied to investigation of the effects of drugs that alter levels of endogenous substrates, the results become even more difficult to interpret. This problem can be obviated by first subjecting the specimen to some procedure that removes endogenous substrates without altering enzyme activity. For example, one convenient technique that has been employed with success for this purpose is Sephadex gel-filtration.[6]

REFERENCES

1. R. J. Levine and A. Sjoerdsma, *Ann. N.Y. Acad. Sci.* **107**, 966 (1963).
2. S. Otsuka and Y. Kobayashi, *Biochem. Pharmac.* **13**, 995 (1964).
3. C. M. McEwen, Jr., *J. biol. Chem.* **240**, 2003 (1965).
4. Y. Kobayashi, *Biochem. Pharmac.* **15**, 1287 (1966).
5. J. V. Hodge, J. A. Oates and A. Sjoerdsma, *Clin. Pharmac. Ther.* **5**, 149 (1964).
6. R. J. Levine and D. E. Watts, *Biochem. Pharmac.* **15**, 841 (1966).