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THIS VOLUME IS DEDICATED TO PROFESSOR J. KNOLL
ON THE OCCASION OF HIS 60TH BIRTHDAY.

PREFACE

This volume is dedicated to Professor Joseph Knoll on the occasion of his 60th birthday.

Dr. Knoll is a graduate of the Budapest University Medical School (now the Semmelweis University of Medicine). He has been working in the Department of Pharmacology since finishing his university studies and was appointed professor and department head in 1962.

The broad range of his scientific activity and his original thinking have made his scientific endeavours extremely fruitful. He is not only outstanding in the area of scientific theories, but he also excels at applying them in practice.

He has devoted himself to extensive research in neuro- and psychopharmacology, a field in which he has developed numerous techniques and new compounds which won him international fame. He has developed the first selective monoamine oxidase inhibitor, deprenyl (Jumex, Elderpyl) which became a drug used world-wide as the prototype of the selective inhibitors of the B type of MAO and is registered in a number of countries as an adjuvant in the therapy of Parkinson's disease.

He has worked out a new group of non-narcotic analgesics (homopyrimidazols) with special spectrum of activity (one member of this group, Probon, is a registered drug in Hungary and in many other countries) and a new family of semisynthetic morphine derivatives, (azidomorphines), with remarkably low tolerance and dependence capacities. Using N-substituted norazidomorphines as new tools, he succeeded in discriminating

between opiate receptors related to cholinergic or adrenergic transmission machinery.

He has analysed the effect of pharmacological modifications of the catecholaminergic and serotonergic tone in the brain and produced new amphetamine structures which act selectively on these systems. These compounds proved to be useful means of demonstrating that the learning ability of rats is positively modulated by the catecholaminergic system and negatively by the serotonergic one. A pharmacologically-induced increase of the serotonergic tone was found to improve processing and retention of information in the rat.

A special field of Professor Knoll's is the study of physiological mechanisms involved in the transformation of the resting state into excitation of the excitable cell membrane. In the course of these investigations he detected a substance in the frog's skin, called celluline, which had a unique influence on the heart cell membrane.

He has recently revealed exciting aspects of the presynaptic mechanisms which modulate transmission. He has detected a small molecular weight substance in human serum (angiohypotensin) which blocks noradrenaline release from the nerve terminals in vascular smooth muscle with considerable selectivity.

From the very beginning of his scientific career he has been deeply involved in the analysis of the mechanisms of higher nervous activity and proposed a new theory to explain drive-motivated behaviour (Theory of Active Reflexes, Akadémiai Kiadó, Budapest, 1969). A recent result of this research was the discovery of satietin, a potent and highly selective anorexogenic glycoprotein in human serum, which seems to behave as the rate-limiting satiety signal in the negative feed-back of food intake.

Joseph Knoll has been widely honoured for his scientific activity, being a member or honorary member of many learned societies, editorial boards of numerous scientific periodicals, and the recipient of a number of awards. He is the member of the Hungarian Academy of Sciences and the German Academy of Natural Sciences (Leopoldina) and doctor honoris causa of the University Medical School, Magdeburg. In 1981, he received the

Semmelweis Prize, the highest recognition awarded in medical science in Hungary and in 1982 he was given the Issekutz Prize, the highest Hungarian award in pharmacology.

Professor Knoll is also an outstanding teacher and has organized a remarkable team to assist him in his work.

This volume contains papers written by his friends and colleagues from all over the world. Most of them present results achieved in those scientific areas which were first opened by Professor Knoll.

The Editors

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Neuropharmacology '85
K. Kelemen, K. Magyar, E. S. Vizi (eds)

SELECTIVE IRREVERSIBLE INHIBITION OF MONOAMINE OXIDASE

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The discovery by Knoll and his colleagues (Knoll et al., 1965; Magyar et al., 1967; Knoll et al., 1968; Knoll and Magyar, 1972) that the irreversible inhibition of monoamine oxidase (MAO) by the acetylenic compound 1-deprenyl was dependent upon the substrate used to assay for enzyme activity, together with findings of substrate-selective inhibition by other compounds such as clorgyline (Johnston, 1968; Hall et al., 1969), pargyline (Fuller, 1972) and Lilly 51641 (Fuller, 1968 & 1972), gave considerable impetus to studies into the biochemical and clinical properties of monoamine oxidase inhibitors. Since this pioneering work, a large number of other monoamine oxidase inhibitors have been shown to be substrate selective (for reviews, see Achee et al., 1977; Fowler, 1982), and there has been a number of studies into structure-activity relationships for different series of both reversible and irreversible selective inhibitors (see e.g. Florvall et al., 1978; Dostert et al., 1982; Mantle et al., 1976; Knoll et al., 1978; Kalir et al., 1981; Tipton et al., 1982a). In this chapter, some factors involved in the substrate-selective irreversible inhibition of monoamine oxidase and their possible applications to the design of such compounds will be disucssed.

SUBSTRATE-SPECIFICITIES OF MONOAMINE OXIDASE -A AND -B

The use of the selective inhibitor clorgyline led Johnston (1968) to propose the existence of two forms of monoamine oxidase which he termed the A and B forms. The former was sensitive to inhibition by very low concentrations (around nanomolar) of clorgyline and was active towards 5-hydroxytryptamine (5-HT) whereas the latter was relatively insensitive to this inhibitor and active towards benzylamine. On this nomenclature deprenyl (Knoll and Magyar, 1972) and to a lesser extent pargyline (Fuller, 1972) were found to be selective inhibitors of MAO-B whereas Lilly 51641 behaved rather similarly to clorgyline (Fuller, 1968, 1972).

Subsequent work with these inhibitors has allowed the specificities of the two forms of MAO (see e.g. Houslay and Tipton, 1974; Neff and Yang, 1974; Suzuki et al., 1981; Tipton et al., 1982b) and their distributions in different organs to be studied in detail. As shown in Table 1, the relative proportions of the two forms vary considerably between different organs and animal species.

Table 1. Proportions of MAO-A and -B in some mammalian organs

MAO-A Predominant	MAO-A and -B present in roughly similar amounts	MAO-B Predominant
Human Placenta	Human Brain	Human Platelet
Rat Heart	Rat Brain	Ox Liver
Rat Spleen	Rat Liver	Pig Heart, Liver & Brain
Guinea Pig Intestine	Mouse Brain	Mouse Liver

(Data from: Fowler et al., 1978; Hall et al., 1969; Lyles and Greenawalt, 1978; Squires, 1972; Tipton et al., 1976; Zeller et al., 1979).

The data in Fig. 1 indicate that most substrates interact with both forms of MAO, but that often one form has a lower K_m value and a higher V_{max} value than the other form towards a particular substrate. Thus, in tissues where both MAO-A and -B are present in roughly similar amounts, 5-HT (at low concentrations) is a substrate for MAO-A alone, 2-phenethylamine (at low concentrations) is a substrate for MAO-B alone, whereas tyramine and tryptamine are substrates for both forms of MAO (for further discussion, see Tipton et al., 1982b). The different substrate-specificities of MAO-A and -B can be thus used to study the behaviour of irreversible inhibitors, although the interpretation of several earlier studies may have been complicated by failure to appreciate the overlapping specificities of the two forms.

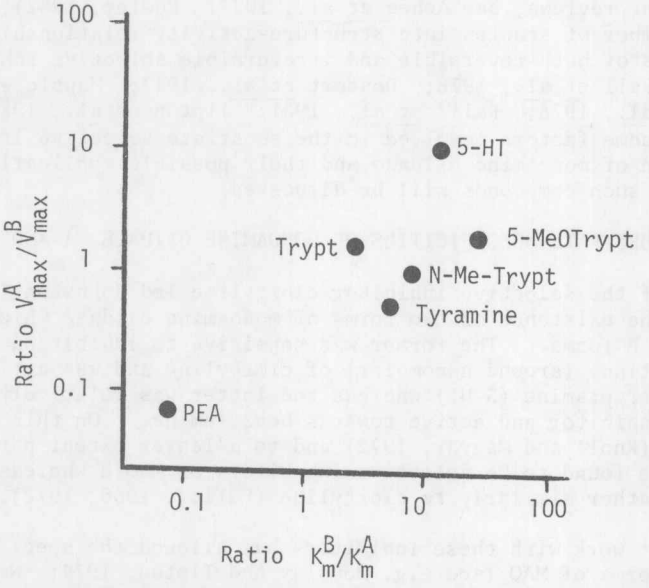

Fig. 1 The substrate specificities of the two forms of rat liver monoamine oxidase. The data were plotted from Suzuki et al., (1981) and Tipton et al., (1982b). The following abbreviations are used: 5-MeOTrypt, 5-methoxytryptamine; N-Me-Trypt, N-methyltryptamine; PEA, 2-phenethylamine; Trypt, tryptamine.

SELECTIVE IRREVERSIBLE INHIBITION OF MAO

The acetylenic inhibitors of monoamine oxidase, a group to which pargyline, clorgyline and l-deprenyl belong, have been studied more extensively than other types of irreversible inhibitors of this enzyme. They have been shown to act as enzyme-activated irreversible inhibitors ('k_{cat}' or 'suicide' inhibitors) of the enzyme (see Hellerman and Erwin, 1968; Abeles and Maycock, 1976; Singer, 1979; Fowler et al., 1982). Such inhibitors act by forming an initial non-covalent complex (E.I) with the enzyme, analogous to the enzyme-substrate complex. Reaction within this complex subsequently leads to the formation of the irreversibly inhibited enzyme species (E-I). This reaction involves the activation of the inhibitor by the enzyme and subsequent adduct formation. The pathway of the inhibition can thus be represented as:

$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} E.I \xrightarrow{k_2} E\text{-}I$$

The values of the parameters K_i and k_2 for the inhibition of rat liver MAO-A and -B by clorgyline, l-deprenyl and pargyline have been determined (Fowler et al., 1982), and are summarised in Table 2.

Table 2. Kinetics of the inhibition of monoamine oxidase -A and -B by clorgyline, l-deprenyl and pargyline

	Clorgyline		l-Deprenyl		Pargyline	
	MAO-A	MAO-B	MAO-A	MAO-B	MAO-A	MAO-B
K_i (μM)	0.05	60	40	1	15	2
k_2 (min^{-1})	>0.76	0.06	0.14	>0.99	0.20	0.20

The data shown in Table 2 indicate that the selectivity of the different inhibitors depends on the relationship between their affinities for the two forms of the enzyme, measured by the dissociation constant K_i, and the relative rates of reaction within this complex, governed by k_2, to give the irreversibly inhibited species. In the case of pargyline, the selectivity is due solely to a greater affinity for non-covalent interaction with MAO-B, since the k_2 values are similar for both enzyme forms. A higher k_2 value with MAO-B reinforces the greater affinity of l-deprenyl for this form of the enzyme, and the concerted action of these two factors is even more evident in the selective inhibition of MAO-A by clorgyline.

The dependence of the selective inhibition of MAO on these two different kinetic factors must be taken into account in attempts to design selective irreversible inhibitors. For example, although there are a large number of selective reversible inhibitors of MAO, chemical modification of these compounds to incorporate an acetylenic group may not necessarily preserve the selectivity, since the k_2 values for reaction with each of the forms may oppose the affinity effects (for further discussion, see Tipton et al., 1983a).

Since the enzyme-activated irreversible inhibitors behave as substrate analogues in binding and being transformed by the enzyme, it would not be surprising to find that in some cases transformation of the inhibitor could result in its release as a product either by slow breakdown of the covalent