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MONOAMINE OXIDASE INHIBITING PROPERTIES OF AB-15—COMPARISON WITH TRANYLCYPROMINE, NIALAMIDE AND PARGYLINE

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Abstract—AB-15* is a potent inhibitor of MAO both *in vitro* and *in vivo*. Single oral application produces long lasting MAO inhibition. AB-15 reverses the action of reserpine and potentiates the 5-HTP induced rise in rectal temperature in mice while having a slight effect on the blood pressure and heart rate responses to tyramine in cats. The *in vitro* studies on the deamination of different amines, indicate that its inhibiting effect is selective, and depends markedly on the substrate used.

IN THE search for potential beta-blocking agents a group of compounds possessing potent MAO inhibiting activity has been discovered. AB-15 was selected from these compounds and its biochemical and pharmacological properties studied in detail.

EXPERIMENTAL

Manometric determinations of MAO activity with tryptamine, dopamine and noradrenaline were carried out using dialysed tissue homogenates as enzyme preparation. The reaction mixtures contained 10^{-2}M of substrate and 10^{-7}–10^{-2}M of inhibitor. The enzymic reactions were run as previously described.[1]

Spectrophotometric determinations of MAO with serotonin and tyramine were made using the methods of Zile[2] and Udenfriend[3] respectively. The inhibitors were used in a concentration range of 10^{-7}–10^{-2}M and substrates in a range of 6×10^{-4}–10^{-3}M. Non-dialysed tissue homogenates were used as enzyme preparations.

Volumetric determination of MAO activity with tryptamine was carried out according to the method of Kappeller-Adler.[4] 10^{-2}M tryptamine and 200–500 mg dialysed tissue homogenate were added to the reaction mixtures.

The tissues were homogenized in 0·1 M phosphate buffer, pH 7·4, using a cell disintegrator, BIOMIX (MIM, LABOR, Budapest).

Human organs were obtained from autopsy, 5–10 hr after death, from individuals of both sexes aged 50–80; causes of death were pneumonia and circulatory disorders.

For the study of MAO activity *in vivo*, white male rats (160–180 g) and male guinea-pigs (400–500 g) were used. Inhibitors were given orally, 16 hr prior to decapitation.

* Abbreviations used: AB-15; 1-meta-aminophenyl-2-cyclo-propylamino-ethanol-dihydrochloride, white crystals, Mw: 265.2 5-HTP: 5-hydroxy-tryptophand; DMPEA: 3, 4 dimethoxyphenylethyl amine MAO: monoamine oxidase.

TABLE 1. COMPARISON OF THE CONCENTRATIONS OF AB-15, TRANYLCYPROMINE, NIALAMIDE AND PARGYLINE, REQUIRED TO PRODUCE 50 PER CENT INHIBITION IN THE DEGRADATION OF SEROTONIN

Inhibitors	I_{50} values: Ml.							
	Rat		Guinea-pig		Mice		Human	
	Brain	Liver	Brain	Liver	Brain	Liver	Brain	Liver
AB-15	$4{\cdot}0 \times 10^{-6}$	$5{\cdot}0 \times 10^{-6}$	$3{\cdot}0 \times 10^{-6}$	$1{\cdot}0 \times 10^{-5}$	$4{\cdot}0 \times 10^{-6}$	$2{\cdot}0 \times 10^{-5}$	$2{\cdot}5 \times 10^{-6}$	$7{\cdot}0 \times 10^{-6}$
Tranylcypromine	$1{\cdot}2 \times 10^{-6}$	$1{\cdot}2 \times 10^{-6}$	—	—	—	—	$6{\cdot}0 \times 10^{-6}$	—
Nialamide	$5{\cdot}7 \times 10^{-6}$	$1{\cdot}2 \times 10^{-5}$	—	—	—	—	—	—
Pargyline	$2{\cdot}0 \times 10^{-6}$	$3{\cdot}8 \times 10^{-6}$	—	—	—	—	—	—

I_{50} (50 per cent inhibition) values were determined graphically.

Serotonin measurements were carried out using the method of Phillip.[5] The reversal of reserpine facilitation of convulsion induced by pentylenetetrazol and potentiation of 5-HTP effect in mice were measured according to Glylis.[6] The potentiation of the tyramine effects was determined in cats, spinalized according to Burn.[7]

Acute toxicity studies were carried out in rats and in mice. The animals were kept in metal cages, ten mice or eight rats per cage. The LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[8]

Tranylcypromine H_2SO_4 and pargyline HCl were kindly supplied by the Smith, Kline and French Laboratories and by the Abbot Laboratories. Nialamide HCl was the product of the United Works of Pharmaceutical and Dietetic Products (Budapest).

RESULTS

MAO inhibition in vitro

A comparison of the MAO inhibiting effect of AB-15 (Fig. 1), with that of the known inhibitors, is shown in Table 1. AB-15 appears effective at concentrations as low as 10^{-5}–10^{-6}M depending on the source of the enzyme.

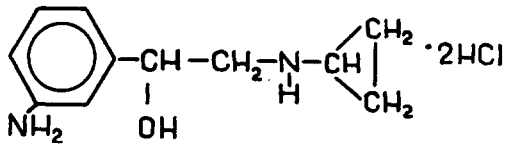


FIG. 1. Chemical structure of AB-15.

TABLE 2. COMPARISON OF THE CONCENTRATION OF AB-15, TRANYLCYPROMINE, NIALAMIDE AND PARGYLINE REQUIRED TO PRODUCE 50 PER CENT INHIBITION IN THE DEGRADATION OF DIFFERENT AMINES

Inhibitors	I_{50} values: Ml.				Range of I_{50} values
	Tryptamine	Tyramine	Dopamine	Noradrenaline	
AB-15	$1{\cdot}2 \times 10^{-2}$	$6{\cdot}0 \times 10^{-5}$	$1{\cdot}0 \times 10^{-4}$	$3{\cdot}7 \times 10^{-5}$	325
Nialamide	$6{\cdot}0 \times 10^{-5}$	$6{\cdot}6 \times 10^{-6}$	$5{\cdot}5 \times 10^{-5}$	$1{\cdot}2 \times 10^{-5}$	10
Tranylcypromine	$7{\cdot}0 \times 10^{-6}$	$1{\cdot}6 \times 10^{-5}$	$2{\cdot}0 \times 10^{-5}$	$3{\cdot}0 \times 10^{-6}$	16
Pargyline	$4{\cdot}0 \times 10^{-6}$	$7{\cdot}2 \times 10^{-7}$	$3{\cdot}2 \times 10^{-6}$	$5{\cdot}0 \times 10^{-7}$	8

Source of enzyme: rat brain homogenate.

The MAO inhibitions by AB-15 and by the reference agents, using different amines as substrate (10^{-2}M of each), are shown in Table 2. Significant differences in the I_{50} values of AB-15 could be observed. Approximately three hundred times higher concentration of AB-15 is required to block the oxidation of tryptamine as compared to the concentration needed to inhibit the deamination of noradrenaline. The I_{50} values of tranylcypromine, nialamide and pargyline slightly varied with the substrates used.

MAO inhibition in vivo

The ED_{50} values of AB-15 and the reference agents are presented in Table 3. The

compounds were given orally to the animals and the MAO activity was measured immediately after decapitation. The ED_{50} values show that the action of AB-15 is more pronounced in guinea-pigs than in rats. The inhibitions produced by AB-15 and by tranylcypromine were closely equal in guinea-pigs while tranylcypromine showed twice or three times higher potency than AB-15 in rats.

Single oral application of AB-15 (18·8 μmoles/kg) produced long lasting MAO inhibition (Fig. 2). The inhibition lasted much longer in the brain than in the liver.

TABLE 3. COMPARISON OF THE EFFECTIVE DOSES OF AB-15, TRANYLCYPROMINE AND NIALAMIDE, REQUIRED TO PRODUCE 50 PER CENT INHIBITION IN THE DEGRADATION OF SEROTONIN IN RATS AND IN GUINEA-PIGS

Inhibitor	ED_{50}: μmoles/kg					
	Rat			Guinea-pig		
	Brain	Liver	Brain/Liver	Brain	Liver	Brain/Liver
Nialamide	37·0	8·5	4·35	—	—	—
Tranylcypromine	7.0	7·4	1·00	3·0	5·4	0·57
AB-15	15·4	11·5	1·34	3·8	7·6	0·50

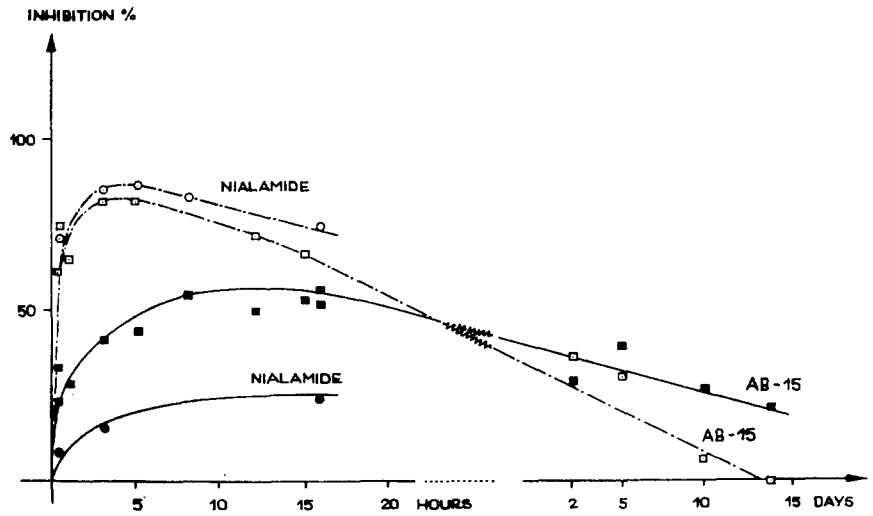


FIG. 2. Duration of the actions of AB-15 and nialamide on MAO activity measured by the degradation of serotonin.

Unbroken and broken lines represent values obtained with brain homogenate and with liver homogenate respectively. Rats received 18·8 μmoles/kg of AB-15 and 15 μmoles/kg of nialamide.

The increased level of serotonin produced by AB-15 and by tranylcypromine in whole rat brain is shown in Table 4. The compounds were given daily for 7 days and rats were sacrificed 24 hr after the last treatment.

AB-15 produced lower increase in the level of serotonin than tranylcypromine after 7 days treatment, however single doses of the compound increased the concentration of serotonin approximately to the same extent.

Reversal of reserpine effect

The action of AB-15 on reserpine facilitation of convulsion induced by pentylenetetrazol is demonstrated in Fig. 3. and Table 5.

The duration of its action was examined by pretreating mice with the inhibitor several days before reserpine challenge and the lethal doses of pentylenetetrazol were determined after reserpine administration. Figure 3 shows that AB-15 markedly reversed the action of reserpine also in that case when animals received it 7 days prior to reserpine.

In order to compare the effect of AB-15 with that of the known inhibitors, AB-15 and tranylcypromine were given 3 hr, nialamide and pargyline 18 hr before and

TABLE 4. THE EFFECTS OF AB-15 AND TRANYLCYPROMINE ON THE LEVEL OF SEROTONIN IN RAT BRAIN

Inhibitor	Serotonin (μg/g tissue)	Increase (%)
Control	0·52 ± 0·10	—
AB-15 (18·8 μmoles)	0·83 ± 0·09	59·6
Tranylcypromine (4 μmoles)	0·94 ± 0·12	80·7

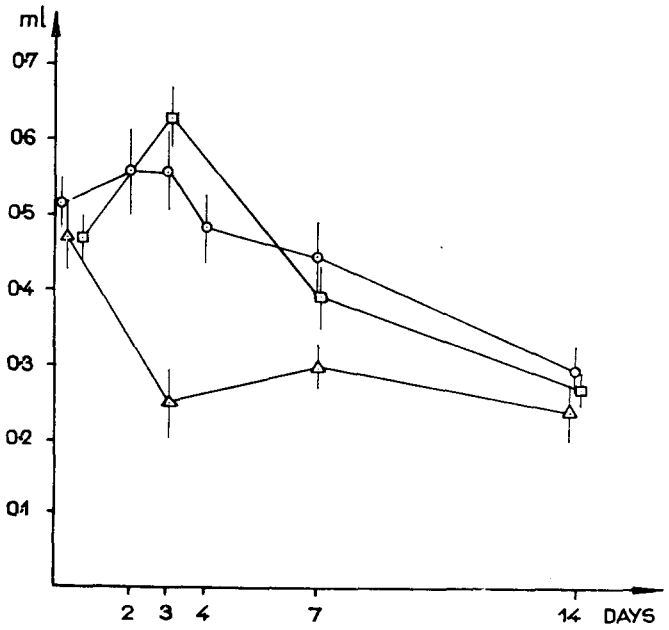


FIG. 3. Duration of the reversal actions of AB-15, tranylcypromine and nialamide on the reserpine facilitation of the convulsive effect of pentylenetetrazol. Abscissa: ml of pentylenetetrazol consumed. Ordinate: pretreatment in days. ○, △, □ 18·8 μmoles/kg of AB-15, 9·4 μmoles/kg of tranylcypromine and 30 μmoles/kg of nialamide respectively.

TABLE 5. REVERSAL OF THE RESERPINE FACILITATION OF THE CONVULSIVE EFFECT OF PENTYLENETETRAZOL BY AB-15, NIALAMIDE, PARGYLINE AND TRANYLCYPROMINE IN MICE

Treatment	Doses mg/kg p.o	Number of animals	ml of pentylene tetrazol consumed	Inhibitor doses in μmoles/kg required to produce 50% effect in antagonizing the action of reserpine
Distilled water	20	18	0·59 ± 0·03	
Reserpine	5	24	0·18 ± 0·01	
Nialamide + reserpine	2·5	9	0·20 ± 0·03	
	5·0	19	0·40 ± 0·03	14·7
	10·0	8	0·47 ± 0·03	
Pargyline + reserpine	50·0	10	0·31 ± 0·03	
	100·0	6	0·57 ± 0·06	—
Tranylcypromine + reserpine	0·25	9	0·21 ± 0·02	
	0·50	9	0·30 ± 0·02	
	1·00	5	0·38 ± 0·04	
	2·00	7	0·47 ± 0·04	4·7
	4·00	9	0·67 ± 0·06	
AB-15 + reserpine	0·5	12	0·21 ± 0·02	
	1·0	20	0·33 ± 0·033	6·5
	2·5	21	0·41 ± 0·03	
	10·0	16	0·51 ± 0·04	
	10·0	8	0·52 ± 0·06	

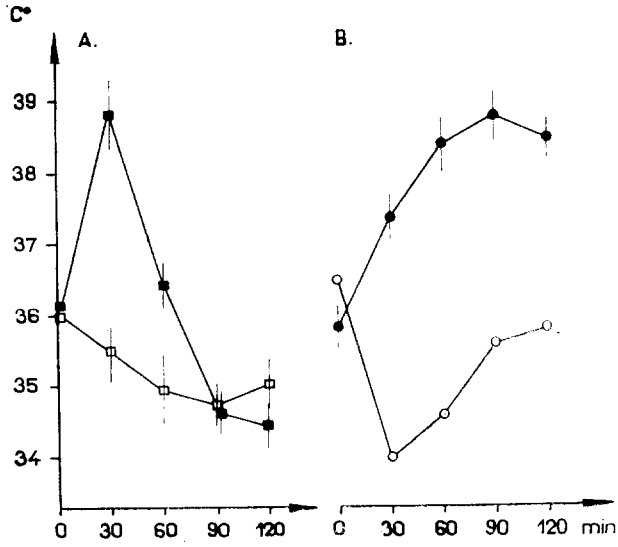
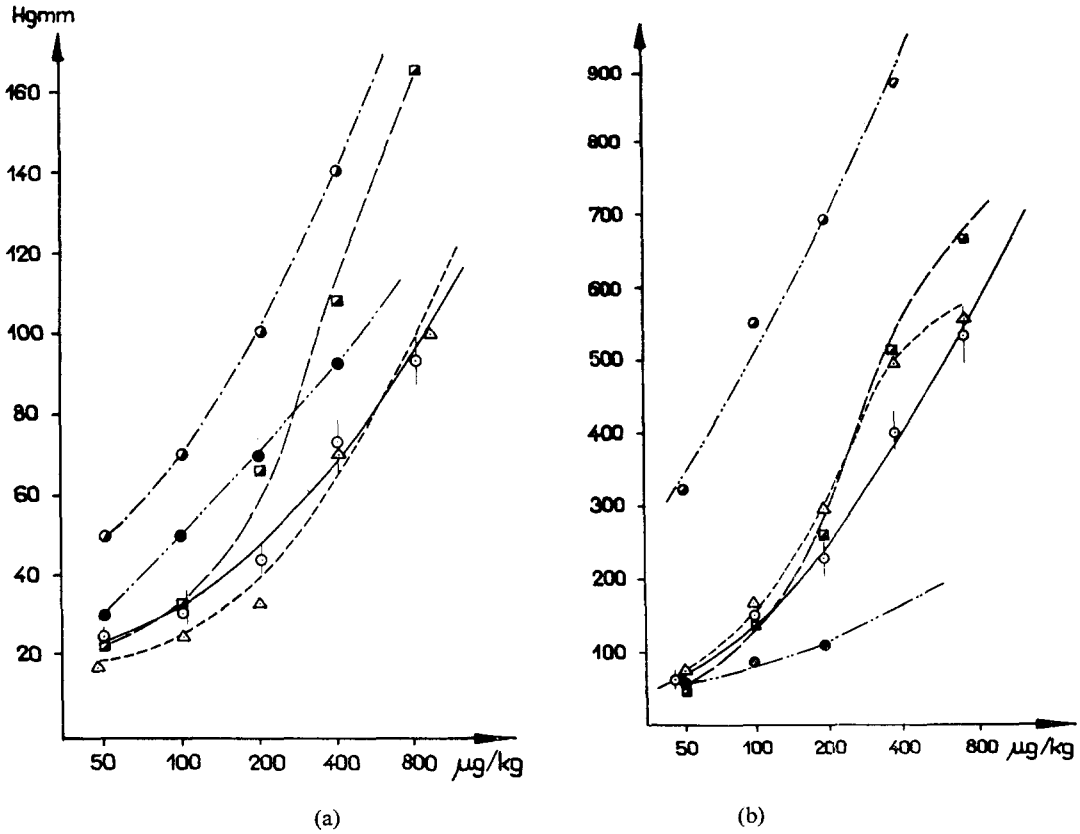


FIG. 4. Potentiation of 5-HTP induced rise in body temperature by AB-15, and tranylcypromine. Abscissa: time after 5-HTP administration. Ordinate: rectal temperature. □. ■. ○. ● 15 μmoles/kg of AB-15; 22·6 μmoles/kg of AB-15; 8·3 μmoles/kg of tranyl-promine and 16·6 μmoles/kg of tranylcypromine respectively.



(a) (b)

FIG. 5(a). The actions of AB-15 and tranylcypromine on the blood pressure response to tyramine. Abscissa: tyramine in μg/kg. Ordinate: increase in blood pressure. Curves were obtained 30 min after the i.v. administration of the inhibitors. ○, ◔, ●, ◪, △: controls, 2·1 μmoles/kg and 16·6 μmoles/kg of tranylcypromine, 7·5 μmoles/kg and 30 μmoles/kg of AB-15 respectively.

FIG. 5(b). The actions of AB-15 and tranylcypromine on the heart rate response to tyramine Abscissa: tyramine in μg/kg. Ordinate: total increase in heart rate during one reaction. Different signs represent the values obtained with those doses of the inhibitor as in Fig. 5(a).

pentylenetetrazol 3 hr after the reserpine administration. The doses of the inhibitors needed to reverse up to 50 per cent the action of reserpine were determined.

AB-15 shows two to three times greater potency than nialamide and closely equal effect with tranylcypromine (Table 5).

Potentiation of the actions of 5-HTP and tyramine

AB-15 showed great ability to potentiate the 5-HTP-induced increase of the rectal temperature in mice. A remarkable increase occurred at the dose of 22·6 μmoles/kg of AB-15 and 16·6 μmoles/kg of tranylcypromine (Fig. 5).

The actions of AB-15 and tranylcypromine on the blood pressure and on the heart

rate responses to tyramine are shown in Figs. 5(a) and (b). Thirty μmoles/kg of AB-15 produced no significant effect on it; 2 μmoles/kg of tranylcypromine markedly potentiated, while 4 μmoles/kg markedly reversed the effects of tyramine in cat.

Acute toxicological studies

The acute lethal doses of AB-15 compared to those of the known inhibitors of MAO are summarized in Table 6. Values were developed 24–28 hr after a single treatment.

AB-15 shows twice lower toxicity than nialamide orally and five times lower toxicity than tranylcypromine with parenteral routes in mice.

TABLE 6. ACUTE TOXICITY VALUES OF NIALAMIDE, TRANYLCYPROMINE AND AB-15 IN MICE AND RATS

Compounds	LD_{50} mg/kg					
	Rats			Mice		
	i.v.	i.p.	orally	i.v.	i.p.	orally
Nialamide	—	—	—	120	435	590
Tranylcypromine	—	—	—	—	91	—
AB-15	390	710	3250	260	470	1060

DISCUSSION

Present biochemical data indicate that AB-15 is a potent MAO inhibitor both *in vitro* and *in vivo*. On the basis of the I_{50} values, measured with serotonin substrate, AB-15 is more effective than nialamide and slightly less effective than tranylcypromine. Its effect is more pronounced on the brain amine oxidase that on the liver. The *in vivo* experiments suggest that the inhibition by AB-15 varies with the species. AB-15 was as potent as tranylcypromine in guinea-pigs and less potent in rats. Its action, especially in the brain was long lasting. The degree and also the type[9] of inhibition produced by AB-15 varies with the substrate used. A concentration three hundred times greater than that required to block the oxidation of noradrenaline or serotonin was necessary to inhibit that of tryptamine.

Gorkin[10] reported that harmine and some tricyclic dyes are more potent as inhibitors of the deamination of serotonin than of that of tryptamine. Recently, Squires[11] and also Fuller[12, 13] reported new inhibitors producing different effects on the degradation of different amines. The authors suggest that the selective inhibition is due to the multiplicity of the enzyme.

Our previous studies[14] with diethyltryptamine and its derivatives suggested that steric factors are responsible for the differences existing between I_{50} values measured on the degradation of serotonin and tryptamine. This explanation was first introduced by Zeller.[15] Recently, significant differences in the sensitivities towards cyanide, and also in the activity distributions of brain MAO-s, have been shown by our studies on the oxidation of serotonin and DMPEA in different brains.[16] Present data obtained with AB-15 indicate that MAO inhibition strikingly depends on the substrates, and also on the enzyme preparations used. It has to be assumed (cf. Refs. 17, 18) that the

forms of MAO vary from one anatomical area to another and also from one organ to another. Presumably, the structure of the different MAO depends on the function of the enzyme. The problem whether the differences in MAO-s are due to different subfragments of the enzyme or if different single enzymes are responsible for the deaminations of the different amines is not known.

AB-15 exhibited higher potency in the reserpine reversal and also in the 5-HTP potentiation tests than that in potentiating the actions of tyramine. The compound was two to three times more potent than nialamide and slightly less potent than tranylcypromine in antagonizing reserpine facilitation of pentylene-tetrazol. A similar relationship between AB-15, nialamide and tranylcypromine was found for potentiation of the 5-HTP induced hyperthermia in mice. Reversal by tranylcypromine which occurred at the higher doses of the inhibitor, does not appear to be related to MAO inhibition. Acute toxicity studies show that AB-15 is less toxic than nialamide or tranylcypromine.

Fuller,[13] suggests that the clinical usefulness of MAO inhibitors in the depressive states depends largely on the ability to block the oxidation of serotonin. Inhibition of the deamination of other amines, especially tyramine might produce undesirable side effects during the treatment. Inhibitors showing proper selectivity, such as AB-15, could be clinically more useful than the other known inhibitors of MAO.

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